FINAL REPORT

ENVIRONMENTALLY SENSITIVE TECHNIQUES IN GOLF COURSE MANAGEMENT: A MODEL STUDY AT THE OCEAN COURSE, KIAWAH ISLAND, S.C.

Submitted to:

The United States Golf Association
Environmental Research Committee
USGA Green Section
Golf House
Far Hills, New Jersey 07931

Submitted by:

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March 1994

EXECUTIVE SUMMARY

The game of golf continues to increase in popularity thus increasing the demand for high quality playing surfaces. Simultaneously, environmental issues associated with golf course development and management are becoming more complex. For instance, turf management practices and pesticide use are important to achieve the quality of playing surface demanded by golfers. Yet, certain management practices can create environmental damage or negative impacts to fish and wildlife. Situations such as large die-offs of waterfowl from pesticide use on golf courses on Long Island Sound created the perspective that golf courses are bad for the environment and that management operations of these courses overlooked the need to protect the environment. The United States Golf Association (USGA) through the USGA Environmental Research Committee has pursued a variety of research projects into environmentally sound golf course management. By addressing pesticide use and other issues, they hope to encourage a stronger commitment to environmentally sensitive management techniques associated with golf course operations.

This report presents the results of a model study at the Ocean Course at Kiawah Island, South Carolina. This study provides a paradigm with which to evaluate environmentally sensitive techniques in golf course development and operation. In our original proposal, we proposed to work with the management staff to evaluate various aspects of water quality, pesticide use, wildlife habitat utilization, and other associated variables which must be considered in promotion of a strong environmental ethic for golf course management. This report is a series of chapters that collectively provide an integrated database on environmental issues associated with pesticide and nutrient use in golf course operations. The chapters proceed through an assessment of avian exposure to pesticides used on the course and biochemical assessment techniques used to address this exposure. The environmental chemistry of pesticides used on the Ocean Course was assessed, particularly an evaluation of chlorpyrifos and bendiocarb movement through soil matrices. A full-scale assessment of carbamate application to the course was conducted to assess pesticide movement from turf into lagoons on the course and into fish or plants. The carbamate used was bendiocarb, a carbamate insecticide used for control of mole crickets. The following chapters focus on the risk to water quality from nutrient loading and on the aquatic system at the Ocean Course, addressing issues of fish exposure to pesticides and the potential for cholinesterase inhibition or mortality. The final chapter provides an overview of a survey to assess management practices on South Carolina golf courses. The survey provides information on the variety of insecticide, herbicide, fungicide, and nematicide use in golf course operations. The common names, trade names, generic names, and action of all pesticides mention in chapters 1-8 are listed in Appendix 1.

As envisioned, this model study provides the nucleus for developing an integrated assessment of the interface between wildlife and their use of golf courses, with the potential risk associated with pesticide or nutrient exposure. The Ocean Course at Kiawah Island offered an extraordinary amount of visibility related to this effort. Several stories have appeared in *Golf Course News* and a feature story in *National Wildlife Magazine* that reached more than 5 million people. Segments of the project have been reviewed on CNN News, as well as state and regional television stations in South Carolina. The study has lent itself to three presentations at a 14th Annual Meeting of the Society of Environmental Toxicology and Chemistry (Appendix 2).

Whether golf course operations are balanced with protection of the environment is a strong issue for the next century. The result of this integrated study was the revelation that intensive golf course management operations, including the use of pesticides and fertilizers, result in some environmental costs, but these can be minimized when approached in an environmentally responsible manner. Our research indicates that we should be aware of fish and wildlife present on the golf course, and be specific and timely with the use of certain products to control pests at economic threshold levels. The over-dependency of nutrients in start-up operations on a new golf course may result in degradation of water quality, particularly enhancing those species of plankton and fish that are more acclimated to "eutrophic" conditions.

ACKNOWLEDGEMENT

Although each of the chapters in this report provide acknowledgements for support for those respective efforts, we thought it appropriate to identify a general context of overall types of support that facilitated completion of this integrated study. First, we are indebted for the support received from the United States Golf Association and their encouragement in our research activities. Mr. Jim Snow and Dr. Mike Kenna are particularly acknowledged for providing insight and support in implementation of this very complex experimental design. We would also like to thank members of the U.S.G.A. Green Committee for their input as well. We felt it helped us improve our work considerably.

Additional financial support was contributed to this project from the PGA of America and Monsanto. We appreciated these gifts and the complete support to pursue lines of research that were appropriate for this project on environmental issues associated with golf course management.

We would be remiss to not acknowledge all the support we had from Kiawah Island, particularly those associated with the Ocean Course. First, Chris Cole is acknowledged for accepting the original conceptual plan and helping us in so many ways as we moved through the process. George Frye is also acknowledged for his help on a day-to-day basis on management issues associated with the Ocean Course. Pete Dye, for his courage to design a golf course on such a sensitive ecological zone with environmental stewardship in mind. These gentlemen were tremendous to work with for our students and faculty, and our testimonial that environmental issues can be balanced with golf course management programs in a mutually beneficial manner.

Many other people contributed to this project and it is impossible to identify everyone. However, those key faculty members that were directly involved included; Dr. Mike Hooper, Dr. Steve Klaine, Dr. Tom La Point, and Dr. Carol Weisskopf. We appreciated all of their involvement and input for our graduate students. To implement an integrated study such as this provides a number of difficult coordination details and the faculty and particularly the graduate students showed a lot of flexibility and support for the project's success.

We believe that this project presents a model for integrated biological and chemical study associated with golf course ecosystems. It also creates an experimental design to generate a data base that can truly allow an individuals to conduct their own ecological risk assessment on their respective course. In this regard, one can then make better decisions on benefits/costs associated with

management actions, particularly with the use of agricultural chemicals.

Again, we are indebted to the United States Golf Association for giving us the opportunity to develop this paradigm and share it with the Golf Course Managers Association. We also appreciate the support and involvement of Mr. Bill Roberts and the entire Golf Course Superintendents' Association of America for their input and involvement.

Finally, we acknowledge Bob Schuster and Beth Ann of our staff. Bob has brought national and international publicity to this project including coverage by CNN, NBC, *Golf Course News*, and *National Wildlife Magazine*, among others. Beth Ann has our gratitude for all her efforts in compiling this report.

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INTRODUCTION

The Ocean Course at Kiawah Island, South Carolina, was completed immediately before the Ryder Cup competition in September, 1991. The course is located on Kiawah Island, South Carolina, a barrier island south of Charleston, South Carolina. Many of the golf course holes are ocean front and are integrated into the primary and secondary dune systems. There exists on the course freshwater pond habitats, brackish and saltwater marsh areas, and maritime forest consisting of palmettos, live oaks, and other vegetation normally found on South Carolina coastal barrier islands. The area abounds with wildlife. On any given day one may see a variety of bird species including shorebirds, wading birds, passerines, and raptors. Mammals, reptiles, and amphibians abound as well as an abundance of fish that attract many species of wildlife for feeding. Basically, this area offers world-class golf in one of the most unique ecosystems of the world; a southeastern river estuary flowing into the south Atlantic coastline.

Mr. Pete Dye, the golf course architect, developed the course with many environmentally sensitive concepts in mind. For instance, the golf course irrigation system involves holding ponds to allow reuse of runoff water on the golf course. Essentially, it is a closed system. An attempt was made to leave as much of the native vegetation as possible and only indigenous vegetation has been utilized to develop the golf course. The area is currently a "bird-watchers dream" and a very challenging course for golfing.

Issues of environmental concern related to golf course operations, particularly along the coastal zone of South Carolina have increased in importance in recent years. We recognized the opportunity for a model study on Kiawah Island. Therefore, once this course was completed, the stage was set to manage an environmentally sensitive model to promote research and strong environmental ethics in golf course operations. Careful thought had been placed into the design prior to construction. Our interactions with the maintenance of the course could readily alert us to any potential impact to wildlife. We evaluated the use of certain kinds of pesticides which had the potential to harm wildlife and create negative impacts for a golf course located in such a sensitive coastal zone area.

TIWET scientists have been involved with a variety of wildlife and pesticide issues associated with golf courses over the last decade. We now have a much better understanding of the turf management issues related to pesticide use and the potential impact to wildlife [1]. A study conducted in the Pacific Northwest of the United States revealed a common feeding ecology of the American wigeon (*Anas americana*). The American wigeon utilized golf courses for grazing on turf that had been treated with diazinon, an organophosphate insecticide, resulting in high exposure to the pesticide. A die-off of 85 wigeon occurred after one application [2]. Events such as birds dying on golf courses, creates a negative perception of the impact of golf on the environment; therefore, we wanted to be particularly observant of bird utilization of the Ocean Course at Kiawah Island. We believed that such impacts could be avoided and the promotion of a sound environmental approach to the management of a golf course could be achieved through careful thought and planning.

OBJECTIVES TO THE OCEAN COURSE, KIAWAH ISLAND ENVIRONMENTAL AND GOLF COURSE STUDY

1. To establish a model study at Kiawah Island, South Carolina, Ocean Course to promote environmental issues associated with golf course maintenance.

2. To evaluate wildlife utilization of the course.

3. To assess water quality for pesticide residues and other contaminants which might affect environmental quality on the site.

4. To work with Kiawah Island Golf Course operations to encourage management that would be environmentally sensitive and encourage wildlife utilization of the area, and support environmental design features which recycle water and minimize the use of pesticides.

AN OVERVIEW OF METHODS FOR THE OCEAN COURSE, KIAWAH ISLAND ENVIRONMENTAL GOLF COURSE STUDY

In the development of the Ocean Course, Kiawah Island Golf Course study, TIWET scientists, having been involved with studies on golf courses for over a decade, were familiar with golf course operations and how to integrate their research so as not to disturb operations that were underway at the Kiawah Island course. We were able to integrate smoothly into the Kiawah Island staff operations.

We developed a general assessment of pesticide residues and other potential contaminants to water on-site. The initial pilot work helped us document the current status of the course, wildlife utilization, diversity and abundance, and helped us better define our research plan as the model study developed. TIWET scientists evaluated golf course turf, water, soil, and other environmental matrices for pesticide residues. We worked with the Kiawah Island operations staff to acquire samples from turf, water, and soil to establish baseline information for the use of pesticides and nutrients on the course. All samples, both environmental and biological, collected during the project were transported to TIWET laboratories located at Clemson University, Pendleton, South Carolina. All work was conducted under a strict Quality Assurance/Quality Control Plan with supporting Standard Operating Procedures.

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CHAPTER I

THE OCEAN COURSE
ON KIAWAH ISLAND, SOUTH CAROLINA

THE OCEAN COURSE ON KIAWAH ISLAND, SC

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INTRODUCTION

Kiawah Island, South Carolina (32E36NN, 80E04NW), lies approximately 20 km southeast of Charleston Harbor (Figure 1). Unlike most other southeastern barrier islands, Kiawah is aligned east to west. It has been prograding (increasing in size in a seaward direction) since construction of a jetty in the Charleston Harbor during the late 1870's. The island is notably stable, although most of the southeast point has formed during the past century.

Kiawah Island encompasses nearly 3,700 hectares of tidal marsh, maritime forest and sand dunes inhabited by abundant wildlife. Over 150 avian species, eight of which are rare or endangered, 18 terrestrial mammalian species and 30 reptile and amphibian species have been observed on Kiawah Island [1]. Brackish ponds and pond margins provide the most critical habitat for these animals.

A residential and resort community, Kiawah Island has developed numerous recreational facilities to date, including four golf courses. The newest of these, the Ocean Course, has received national recognition for demonstrating environmental responsibility. The European-style links course is situated in a sand dune and tidal marsh ecosystem. Designed by Pete Dye, the Ocean Course was constructed with an innovative drainage system which recaptures irrigation runoff. The irrigation and drainage system recycles water and may deter chemicals used on the course from entering adjacent wetlands.

STUDY JUSTIFICATION

Membership in the Professional Golfers' Association (PGA) increased by 50% during the 1980's and the United States Golf Association has more than doubled over the past five years. The National Golf Foundation (NGF) reports nearly 28 million amateur golfers in 1990, twice the number of 15 years ago. Over three thousand new golf courses have been built since 1970 in the United States and NGF predicts the construction of 400 new courses each year throughout this century to accommodate growth in the sport.

Several environmental concerns are associated with the expanding number of golf courses. Large tracts of land are used to construct golf facilities. Frequently, sites are developed in previously untouched rural areas which can be ecologically vulnerable. Wildlife habitat can be lost, disturbed or contaminated. Golf demands high quality playing surfaces, which often require extensive application of fertilizers, fungicides, herbicides and insecticides. These chemicals may directly affect wildlife using the course [2] or enter neighboring aquatic ecosystems and affect wildlife, birds and aquatic species some distance from the course. Increasing environmental awareness of the public and among turf grass professionals requires an evaluation of current management practices.

Misuse of horticultural chemicals has the capacity to damage sensitive ecosystems. Further, irresponsible use of large quantities of chemicals, required to maintain golf courses, can result in ecological disaster. The deaths of hundreds of waterfowl on golf courses can be traced to misuse of the organophosphate insecticide, diazinon [3]. Eighty-five American widgeon (*Anas americana*) died within 45 minutes after grazing on a diazinon-treated fairway in Bellingham, Washington [2]. Cadmium, an extremely toxic heavy metal, is a common component of fungicides used on golf courses [4]. Cadmium has been shown to accumulate in the kidneys and livers of avian species when fed earthworms taken from cadmium-treated turf [5]. Paraquat and atrazine, two widely used herbicides, are also hazardous to certain nontarget animals. Exposure to paraquat, at concentrations lower than those used for weed control, proved lethal to honeybees [6]. Atrazine contamination of aquatic habitats is believed to contribute to the declining number of North American frogs [7].

Many golf facilities create highly attractive environments for wildlife. Water, food and cover are readily available and may increase the potential for pesticide exposure. Yet golf courses need not be a threat to wildlife. Well designed and managed facilities can supply quality habitat, especially in heavily developed areas. Studies by Green and Marshall [8] credit the survival of several British plant and animal species to refuge provided solely by golf course rough areas.

Golf courses contribute a natural park-like setting to urban areas. Outdoor recreation in attractive surroundings is an important part of the allure of golf. Large turfed areas also serve to reduce runoff, recharge groundwater and provide oxygen through photosynthesis.

These environmental aspects of golf course management were taken into consideration during the development of the Ocean Course on Kiawah Island.

KIAWAH ISLAND HISTORY

European settlers discovered the island during the late 17th century, naming it after its inhabitants, the Kiawah Indians. The Vanderhorst family owned the island for most of the next 200 years, cultivating traditional low country crops, such as indigo and cotton. The plantation failed in 1917, when the boll weevil (*Anthonomus grandis*) was introduced to the sea islands.

In 1952, the C.C. Royal Lumber Company purchased Kiawah for $50,000, with plans to harvest the timber growing in the unworked cotton fields. The Royal family and friends used the island as a fishing and hunting retreat until 1974, when it was purchased for $17.4 million by the Kiawah Island Company, a subsidiary of the Kuwait Investment Company.

Led by Charles Fraser, the Sea Pines Company was engaged to plan and manage the island's development. Fraser envisioned a residential and resort community compatible with the natural habitats of Kiawah Island [9]. A $1.3 million, 16 month environmental survey of the island laid the foundation. Scientists from thirteen different disciplines conducted geological, climatic, archeological, botanical and wildlife studies to produce the *Environmental Inventory Of Kiawah Island* [10]. The compilation was used by environmentalists, engineers, architects, horticulturists, land planner's and other development professionals to create the Kiawah Master Land Use Plan, which is still followed today.

The island was purchased for $105 million from the Kuwaitis in 1988 by a group of Charleston entrepreneurs, who formed the Kiawah Resort Association. In 1989, Landmark Land Company, Inc. negotiated control of all island resort properties. Holdings included the inn, conference center, tennis facilities and three existing golf courses, Marsh Point, Turtle Point and Osprey Point. Landmark Land also purchased over 200 acres (75 hectares) of beach front property on the south east tip of the island for the fourth golf course. This land was intended solely for the construction of the Ocean Course. No private residences are planned to border the golf course.

Ocean Course Prior To Construction

Aerial photographs indicate the construction site was primarily sand dunes, thicket and salt marsh. Pre-construction vegetation was similar to habitats elsewhere on the island. Sea oats (*Uniola paniculata*), provide primary cover on sand dunes and are critical to dune stability. Wax myrtle (*Myrica cerifera*), yaupon holly (*Ilex vomitoria*) and stunted live oak (*Quercus virginiana*) compose most of the denser thickets between the dunes and forest edge. The dominant salt marsh vegetation is smooth cord grass (*Spartina alterniflora*). Live oak and palmetto (*Sabal palmetto*) forests border the site to the north.

Although no formal surveys were conducted prior to course construction, wildlife on the construction site were reported to be diverse and abundant. The least disturbed area on the island was a haven for sensitive species, such as osprey (*Panion haiaetus*) and gray fox (*Urocyon cinereoargenteus*). Ibis pond, which borders the course to the north, attracted diverse waterfowl, including the American black duck (*Anas rubripes*) and red-breasted merganser (*Mergus serrator*).

Course Construction

Golf course architect, Pete Dye, proposed a golf course designed to ensure the biotic integrity of the site. In the summer of 1989, Dye and his crew began the project. Dye was present on the site during the entire two years of construction, a practice not shared by most course designers.

Dye generally designs golf courses as they are built and rectifies difficulties as they are encountered. No formal plans were drawn except for basic cart routing designs. Sand dunes were razed and rebuilt as Dye directed from the field. Although this may have allowed for more sensitive development of the site, it is difficult to document the process, as few records were kept during actual construction.

The most unusual architectural feature of the Ocean Course is the drainage system. An extensive underground network of tiles recaptures irrigation water. In 1987 Dye installed a similar system beneath nine holes of the Old Marsh Golf Club in West Palm Beach, Florida. Constructed to protect bordering wetlands from runoff, the Florida golf course drainage system has been shown to be effective. Water samples, taken biannually, give no evidence of chemical leaching into the marsh [11].

The same design is used under the entire Ocean Course; however the chief purpose is for water reclamation. Water pumped to the Ocean Course is first used on the back nine holes, then routed to a holding lagoon for use on the front nine holes. Before the water enters the conduit system, it must pass through several feet of fine sand. The sand acts as a filter, resulting in quality recycled water for irrigation. Though the system may control golf course chemicals from entering nearby wetlands, the original intention was to help meet irrigation requirements. Recycling is an important conservation measure in an area where fresh water is at a premium and water needs are large.

Although unusual, the concept used to design the drainage system is very simple. The course contours slope slightly toward the center of each fairway. This minimizes irrigation runoff away from the course and directs excess water towards conventional drains. Drains and 6-inch (15.2 cm)tiles underlie every tee, green, bunker and fairway. The sublateral 6-inch tiles lead to 12-inch (26.9 cm) lateral tiles. The 12-inch tiles feed into a central channel, made up of subsurface pipes and drainage lagoons. This main channel leads to a holding area. Sublateral tiles under the greens are perforated. Seams at intersections and between tiles are sealed.

The tiles were placed at depths between 1.5 and 5 feet (43 cm and 152 cm), above subsurface saltwater levels. The known range for fresh groundwater is 2.5 to 4 feet (76 cm to 122 cm). A submersible pump station underneath the practice range keeps the lagoon system at about two feet (61 cm) above sea level.

Irrigation water for the back nine originates at the western irrigation lagoon, usually referred to as the "effluent lagoon" (Figure 2). The primary direction of flow is toward the center of the course, where the irrigation lagoons are located. Two lagoons are part of the main channel on the back nine. Discharge from holes 12, 13, 14 and 15 enters the western end of lagoon A through a 15-inch (38.1 cm) pipe. A solid 12-inch tile transports water from the eastern end of lagoon A to lagoon B. Lateral tiles from holes 11 and 16 intersect the main line between the two lagoons. Water from the tenth hole enters below the seventeenth green. A 15-inch pipe drains lagoon B. This main conduit empties into a catch basin beneath the eastern end of the practice range. Drainage from the 18th hole is transported independently through a 12-inch line [12].

The practice range drainage system is composed of three 15-inch, parallel tiles, intersected by 6-inch lateral tiles. This network is perforated and lies within the watertable. Using the pump housed within the catch basin, it is possible to extract groundwater from this point, if necessary. However, the proximity of saline groundwater limits available supplies.

All water draining the back nine holes enters the catch basin beneath the practice range. A pump inside the catch basin is triggered by a flotation device when water levels exceed two feet. The water is then conveyed to the eastern irrigation lagoon, the "recycling lagoon".

Water recaptured from the front nine holes is not suitable for irrigation. Original plans included recycling this water; however, tidal encroachment of the central drainage channel produced salinities intolerable to turf grasses. Dye hopes to eventually locate and strengthen the points of infiltration and collect usable water (Dye, pers. comm.).

On the front nine side of the golf course, there are four drainage lagoons within the main channel (Figure 3). Lagoon F is the most eastern point in the drainage system. From here water is directed towards the center of the course. Drainage water enters the lagoons through lateral 12-inch tiles. Lagoon F receives water from holes 4 and 5, lagoon E from hole 6, lagoon D from holes 2,3,7 and 8 and lagoon C from the first and the ninth holes. The front lagoons are connected by 24-inch tile, through which the return irrigation water flows. An irrigation outlet into lagoon F facilitates circulation, when necessary.

Two 24-inch tiles transport recaptured drainage to a catch basin behind the irrigation lagoons. Water then moves to the settling pond, where breakdown or sedimentation of remaining chemicals may occur. This pond is connected to a catch basin with a pump. Discharge into Willet Pond is triggered at water levels over two feet above sea level.

Initial construction efforts on the Ocean Course focused on enhancing and protecting the fragile ecosystem. Construction provided the opportunity to revive over 30 hectares of previously altered saltwater marsh. Tidal flow was restored to Willet and Ibis Ponds, isolated from Bass Creek since the 1950's by the construction of a logging road.

The completed course covers approximately 91 hectares. All decorative vegetation is native. Nurseries were created to culture plants taken prior to construction. Rows of sand dunes were hand-planted with sea oats and panic grass (*Panicum amarum*). The dunes and extensive roughs were designed to act as barriers between playing areas and the surrounding wetlands. The front nine is bordered by maritime forest and saltwater marsh to the north and slack thickets (primarily of wax myrtle and yaupon) to the east and south. It contains several small groves of live oaks and is bisected by a narrow brackish creek that widens to form a series of small ponds (lagoons C-F). The back nine is bordered to the north and west by a wide brackish creek connecting Ibis and Willet Ponds, and ocean beach and dunes border to the south. There are very few trees on the back nine. Instead, each hole is surrounded by dunes. The clubhouse, practice green, driving range, and turfgrass nursery are located in the middle of the course, separating the front nine from the back nine.

From its inception, the Ocean Course on Kiawah Island was developed with environmental foresight. Proximity to sensitive wildlife habitat and a unique water recycling program make it a possible model for other golf facilities. Initiation of environmentally sensitive techniques may establish new standards for golf turf management.

1991 PILOT STUDY

Pilot study field work began in late July, 1991. Initial inquiries focused on preparing the more-detailed investigations in 1992 and 1993. Priorities included fully understanding the course layout and drainage system design. Wildlife habitats associated with the Ocean Course were identified using aerial photographs and site reconnaissance. Turf management practices (eg., chemical use and irrigation procedures) were documented (Table 1). Potential chemical applications on the Ocean Course were projected using records from the three older golf courses, on Kiawah Island. We recorded wildlife responses, primarily atypical behavior, after pesticide application on the Ocean Course. The existing ecological studies of Kiawah Island were collated. Water samples from Ocean Course lagoons were collected for preliminary chemical analyses. Further details concerning the pilot study follow.

Maps and Aerial Photographs

A diagram of the Ocean Course layout was obtained from Landmark Land Company (Figure 4). It was produced to help coordinate the 1991 Ryder Cup Tournament; however, it suited our purpose well. A blue-print of the Kiawah Island drainage plan was used to locate flood control weirs. This is helpful in identifying outside chemical sources. Topographical maps (USGS 7.5 minute series) of Kiawah and the surrounding quadrangles (Kiawah and James Island) were used to determine major drainage patterns into surrounding estuaries.

Kiawah Resort Associates (KRA) has had aerial photographs of Kiawah Island taken every two or three years since 1975. With their permission, copies of pre-construction (1987) and post-construction (1990) series were purchased from Ayers and Associates, Madison, Wisconsin.

We used the aerial photographs and 1975 survey to identify habitat types associated with the Ocean Course. This information is included in the course description.

Review of Managerial Practices on the Ocean Course

The Director of Golf Course Maintenance oversees management of the four golf courses on Kiawah Island. The Ocean Course turf maintenance crews are led by Course Superintendents. The Director meets with his staff weekly to review management plans and coordinate equipment schedules. The final decision concerning any chemical application rests with the Director.

(1) Irrigation

Most of the water used on the island golf courses is supplied by the Kiawah Island Utilities Company. An effluent transfer pump sends irrigation water to Turtle Point, Osprey Point and the Ocean Course through a single line. Water is extracted at Turtle Point and Osprey Point before the line reaches the Ocean Course. The irrigation water is a combination of wastewater, treated twice at Kiawah Island Utilities Company, potable and well water. Percentages of each vary with rainfall and turf requirements. Further, the effluent component is restricted by local coastal authorities. Cost generally limits the use of potable water. A deep well on the Ocean Course is also a source of irrigation water.

The effluent mixture is stored in the irrigation lagoon and is used only on the back nine holes. The South Carolina Coastal Council does not allow the effluent component to be used on the front side of the course out of concern for local shellfish populations. The recycled effluent mixture from the back nine is permitted because the extensive sand filtering improves water quality.

The Ocean Course is irrigated nightly, over an 8 to 10 hour period. Irrigation begins on the odd-numbered, fairways, tees and approaches on the front nine at 19:00 hours and the odd- numbered back fairways, tees and approaches at 20:00 hours. After four hours, all greens are watered and the even-numbered holes are treated in a similar fashion. The irrigation schedule may vary. For example, the greens are sometimes watered by hand.

(2) Pesticide Use on the Ocean Course

Proximity of marsh and dunes requires that extremely conservative management procedures be followed on the Ocean Course. Wind is a constant consideration and pesticide drift is a concern. If possible, granular products are used instead of liquids to control dispersal and reduce drift. Applications are followed by irrigation to promote infiltration of the chemical. Chemicals of low magnitude (low toxicity) and rapid dissipation are chosen for use (see below). Subsequent treatments are made if the pest problem persists. Repeated applications may be the consequence of staggered hatching of pests or the residual characteristics of the chemical used.

Precautions for human safety are followed and Material Safety Data Sheets were posted for each product used. Records were kept of most applications.

1991 Pesticide Applications and Post-application Wildlife Responses

In 1991, the Ocean Course had been open for play less than six months, and had a very limited pesticide application history. Chemical application records from the Marsh Point, Turtle Point, and Osprey Point facilities were examined to predict pesticide use trends on the Ocean Course.

Orthene7 (common name acephate) and Dursban7 (common name chlorpyrifos) were the two insecticides used on the Ocean Course in Fall 1991 to control sod webworms (Cranbinae subfamily). The postemergent herbicides, Round Up7 and a mixture of Image7 and MSMA7, were spot applied to rough areas almost daily from May to September. Copper-sulfate was added to the irrigation system to prevent waterline clogging.

Although no formal behavioral studies were performed at that time, discussions with turf workers and personal observations indicate insects surface after pesticide application and become ready food for foraging birds.

With excessive exposure, organophosphate insecticides are suspected to cause disturbances in avian behavior and normal physiological function [13,14]. In the days following application of, Orthene, peculiar behavior of gulls was noted. Several appeared lethargic and did not avoid human approach.

One had to be picked up and removed from the playing surface. Personal observations of avian carcasses increased, although no more than three were reported on any day.

The herbicide MSMA7, an arsenate compound, was used for broadleaf control in the sand dunes. The turf crews reported an increase in ghost crab (*Ocypode quadrata*) mortality with MSMA7 use.

Since 1991, chemical requirements of the Ocean Course have evolved as the course has matured (Table 1). Details concerning 1992-1993 pesticide applications and the observed accompanying responses of wildlife are given by Rainwater et al.[15].

ENVIRONMENTAL CONCERNS ON KIAWAH ISLAND

Environmental consciousness is prevalent among island residents. The Kiawah Island Community Association (KICA) maintains nature trails around the island and sponsors a series of nature workshops. Residents are invited to hear local and state authorities discuss wildlife management options. Local efforts to protect the Atlantic loggerhead turtle (*Caretta caretta*) have received national acclaim. Most residents live adjacent to one or another of the island golf facilities and become familiar with management activities. Many residents and visitors harvest fish and shellfish from the island lagoons and are concerned about runoff from the golf courses and parking areas.

All lagoons on the island are under the authority of KICA. Until 1991, water quality testing and aquatic weed control were handled under contract by an outside source. Since that time lagoon management personnel have been acquired. The present lagoon supervisor has implemented biological controls for aquatic weeds, including introduction of sterile grass carp and grass- foraging ducks.

Mosquito control is a problem common to all southern coastal regions. Malathion, an organophosphate, is used by KICA for mosquito fogging in residential areas. Altosid7 is a growth regulator which prevents the emergence of adult mosquitoes. It is used in small bodies of standing water not occupied by fish. During summer months, Charleston County conducts aerial spraying of coastal areas. A variety of chemicals are used including larvicides and malathion. A minor fish kill in one of the course lagoons was reported by turf crews in 1991, following one such event. Spraying occurs only when necessary, usually once each month.

Every six months, groundwater samples from each golf course are monitored by the South Carolina Department of Health and Environmental Concerns. Levels of orthophosphates, ammonia, nitrates, pH, specific conductance and watertable elevation are measured for the deep well and the four monitoring wells on the back side of the Ocean Course. Because no effluent is used directly on the front side, no sampling is required.

Existing Wildlife Studies

Existing nature studies were reviewed to gain insight on wildlife which might use the Ocean Course. The most comprehensive survey to date is the *Environmental Inventory of Kiawah Island*, conducted by the Environmental Research Center, Inc., Columbia, South Carolina [10]. However, wildlife species observed during this survey may not be totally indicative of wildlife species currently present on the Ocean Course area. The survey was conducted fifteen years ago. However, the island habitat was dramatically altered by development and by Hurricane Hugo, which struck the island during the initial surveying for this project on the Ocean Course. During the 1992-1993 field season, we used personal and relayed observations, species' habitat preferences and site characterizations to estimate the susceptibility of non-target species to course construction and management techniques.

Biota Encountered On The Ocean Course During The 1992-1993 Field Seasons

An extensive biotic survey of the Ocean Course and adjacent areas is beyond the scope of this project; however, avian species were monitored. Additionally, incidental observations of terrestrial and semi-aquatic vertebrates provide limited information concerning the effect of the Ocean Course management program on such species.

Avifauna

Seventy-five avian species were observed on the Ocean Course during this study (Table 2). The different habitats located on and adjacent to the course attract a variety of wading birds, shorebirds, waterfowl, passerines, and raptors. Species commonly observed around brackish creeks and ponds include; great blue herons (*Ardea herodias*), great egrets (*Casmerodius albus*), snowy egrets (*Egretta thula*), Louisiana herons (*Florida caerulea*), green herons (*Butorides striatus*), least bitterns (*Ixobrychus exilis*), and common gallinules (*Gallinula chloropus*). Blue-winged teal (*Anas discors*), bufflehead (*Bucephala albeola*), hooded mergansers (*Lophodytes cucullatus*), lesser scaup (*Athaya affinis*), and northern shovelers (*Anas clypeata*) frequent the Ocean Course creeks and ponds during winter months (usually January-March). Red-winged blackbirds (*Agelaius phoeniceus*), common grackles (*Quiscalus quiscula*), and boat-tailed grackles (*Quiscalus major*) are the most ubiquitous species on the course and have been observed in trees, marshes, dunes, and on the golf course turf. Other species commonly observed on turfgrass include laughing gulls (*Larus atricilla*), northern mockingbirds (*Mimus polyglottos*), loggerhead shrikes (*Lanius ludovicianus*), American crows (*Corvus brachyrhynchos*), and killdeer (*Charadrius vociferus*). Eastern kingbirds (*Tyrannus tyrannus*), red-bellied woodpeckers (*Melanerpes carolinus*), eastern bluebirds (*Sialia sialis*), and painted buntings (*Passerina ciris*) are other commonly observed passerines. Predators such as red-tailed hawks (*Buteo jamaicensis*), ospreys (*Pandion haliaetus*), and American kestrels (*Falco sparverius*) commonly perch in trees and fly overhead in search of prey. Willets (*Catoptrophorus semipalmatus*), spotted sandpipers (*Actitis macularia*), American oystercatchers (*Haematopus palliatus*), and black-necked stilts (*Himantopus mexicanus*) can be seen at different times of the year feeding in the salt marshes adjacent to the front nine. Flocks of brown pelicans (*Pelecanus occidentalis*), flying single file, are also a familiar site.

Mammals

Twelve mammal species were observed on the golf course area during this study (Table 3). Most mammals were seen during early and late evening hours, and tracks found on the course in the early morning also indicated nocturnal mammalian activity. Whitetail deer (*Odocoileus virginianus*), raccoons (*Procyon lotor*), and small rodents (*Sigmodon* and *Peromyscus* spp.) were the most commonly observed mammals on the course. Bobcats (*Lynx rufus*), gray foxes (*Urocyon cinereoargentatus*), and opossums (*Didelphis marsupialis*) were rarely seen and were always close to the forest edge. River otters (*Lutra canadensis*) were observed in brackish creeks during winter months. Bats were seen flying over ponds and creeks and along forest edges, but could not be identified visually. However, red bats (*Lasiurus borealis*) and Mexican freetail bats (*Tadarida brasiliensis*) are the only two species previously found on Kiawah Island [10], and are most likely the species we observed.

Reptiles and Amphibians

Ten reptile and four amphibian species were identified on the course during the 1992-93 field seasons (Table 3). The most conspicuous reptile on the course is the American alligator (*Alligator mississippiensis*), which frequents all ponds, creeks, and lagoons on the course, but has also been observed on cart paths, greens, fairways, and dunes. The most common snake observed on the course is the northern black racer (*Coluber constrictor constrictor*), although five other species were identified. Several lizards, turtles, frogs, and toads have also been observed on the course.

Aquatic Organisms

The benthic invertebrate community was sampled several times throughout the field season using a D-frame aquatic net and substrate samplers composed of mesh in wire baskets. All invertebrates were identified to the lowest taxon feasible, by members of this study. Diptera belonging to the midge (Chironomidae) and mosquito (Culicidae) families were commonly found. The order Odonata was represented by the aeshnid dragonfly, *Anax junius* (Drury) and numerous coenagrionid damselfly larvae belonging to the genus *Ishnura*. An inhabitant of special interest is the predacious caddisfly, *Oecetis inconspicua* Complex (order: Trichoptera). Other aquatic insects were also found common (Table 4).

The most numerous crustaceans collected belong to the order Amphipoda, commonly called "scuds" or "sideswimmers". The species most commonly encountered was the ubiquitous *Hyalella azteca* (Saussure) [16]. Common blue crabs (*Callinectes sapidus*) of the order Decapoda were captured by seine in both Ibis and Willet Ponds. Hermit crabs and shrimp were observed in both ponds and in brackish creeks. Marsh crabs (*Sesarma* spp.) and fiddler crabs (*Uca* spp.) are commonly observed along banks of tidal marsh, and ghost crabs are abundant among the dunes on the back nine.

Residing fish species were captured by seine and identified. Eleven species, including killifishes, sleepers, drums, gobies, silversides, mullets, and carp were found to inhabit the creeks and ponds on the course (Table 5).

Flora

Plant life along the fringes of the golf course has changed very little subsequent to construction. Great care was taken to leave these areas in their existing state, thus yaupon holly, live oak and palmetto still dominate woody areas on the front nine and the northern border of the back nine. Emergent vegetation in adjacent salt marshes, Willet and Ibis Ponds and many lagoons on the course includes smooth cord grass and cat-tails (*Typha lattafolia*). Widgeon grass (*Rupia maritima*) is the predominant submerged aquatic macrophyte in course lagoons and water-ways. The dunes are stabilized by sea oats and panic grass.

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Table 1. Information regarding insecticide applications on the Ocean Course from August 1991-August 1993.

Date	Chemical	Common Name	% Active Ingredient	Formulation	App. Rate (lbs/acre)	App. Method	Target Area
08/28/91	Orthene7	Acephate	75	liquid	5	spray	fairways
08/29/91	Orthene7	Acephate	75	liquid	5	spray	greens
08/29/91	Orthene7	Acephate	75	liquid	5	spray	approaches
08/30/91	Dursban7	Chlorpyrifos	1.3	granular	65	broadcast	greens
09/03/91	Dursban7	Chlorpyrifos	1.3	granular	65	broadcast	greens, approaches
09/04/91	Dursban7	Chlorpyrifos	1.3	granular	65	broadcast	fairways
09/05/91	Dursban7	Chlorpyrifos	1.3	granular	65	broadcast	practice range
09/10/91	Orthene7	Acephate	75	liquid	5	spray	slopes
09/11/91	Orthene7	Acephate	75	liquid	5	spray	greens, approaches
09/12/91	Orthene7	Acephate	75	liquid	5	spray	greens, approaches
09/13/91	Orthene7	Acephate	75	liquid	5	spray	greens, approaches
09/14/91	Orthene7	Acephate	75	liquid	5	spray	greens, approaches
09/15/91	Orthene7	Acephate	75	liquid	5	spray	greens, approaches
09/18/91	Orthene7	Acephate	75	liquid	5	spray	all slopes
05/27/92	Dursban7	Chlorpyrifos	1.3	granular	65	broadcast	greens, approaches
05/28/92	Dursban7	Chlorpyrifos	1.3	granular	65	broadcast	greens, approaches
05/28/92	Orthene7	Acephate	75	liquid	5	chemigation[a]	front 9
05/29/92	Orthene7	Acephate	75	liquid	5	chemigation	back 9

Table 1. (Continued, p. 2)

Date	Chemical	Common Name	% Active Ingredient	Formulation	App. Rate (lbs/acre)	App. Method	Target Area
07/03/92	Dursban7	Chlorpyrifos	1.3	granular	65	broadcast	greens, approaches
08/31/92	Orthene7	Acephate	75	liquid	5	chemigation	front 9
09/01/92	Orthene7	Acephate	75	liquid	5	chemigation	back 9
09/28/92	Orthene7	Acephate	75	liquid	5	chemigation	front 9
09/29/92	Orthene7	Acephate	75	liquid	5	chemigation	back 9
05/10/93[b]	Turcam7	Bendiocarb	2.5	granular	120	broadcast	all turf
06/17/93	Nemacur7	Fenamiphos	10	granular	100	broadcast	greens, slopes
07/07/93	Turcam7	Bendiocarb	2.5	granular	120	broadcast	approaches, tees, nursery, clubhouse
07/14/93	Turcam7	Bendiocarb	2.5	granular	120	broadcast	greens
08/05/93	Orthene7	Acephate	75	liquid	5	chemigation	front 9
08/06/93	Orthene7	Acephate	75	liquid	5	chemigation	back 9

[a]Chemigation is a process by which a chemical (in this case an insecticide) is applied through an irrigation system

[b]Approximate date

Table 2. Species codes, vernacular, and scientific names of birds observed on the Ocean Course during this study (November 1991-August 1993).

Code	Vernacular	Scientific name
AMBI	American bittern	*Botaurus lentiginosus*
AMCO	American coot	*Fulica americana*
AMCR	American crow	*Corvus brachyrhynchos*
AMOY	American oystercatcher	*Haematopus palliatus*
AMRO	American robin	*Turdus migratorius*
BASW	Barn swallow	*Hirundo rustica*
BEKI	Belted kingfisher	*Megaceryle alcyon*
BBPL[a]	Black-bellied plover	*Pluvialis squatarola*
BCNH	Black-crowned night heron	*Nycticorax nycticorax*
BNST	Black-necked Stilt	*Himantopus mexicanus*
BLSK	Black skimmer	*Rynchops niger*
BLJA[b]	Bluejay	*Cyanocitta cristata*
BWTE	Blue-winged teal	*Anas discors*
BTGR	Boat-tailed grackle	*Quiscalus major*
BOGU	Bonaparte's gull	*Larus philadelphia*
BHCO	Brown-headed cowbird	*Molothrus ater*
BRPE	Brown pelican	*Pelecanus occidentalis*
BRTH	Brown thrasher	*Toxostoma rufum*
BUFF	Bufflehead	*Bucephala albeola*
CAEG	Cattle egret	*Bubulcus ibis*
CHSP	Chipping sparrow	*Spizella passerina*
CWWI[b]	Chuck-will's-widow	*Caprimulgus carolinensis*

Table 2. (Continued, p. 2)

Code	Vernacular	Scientific name
CLRA	Clapper rail	*Rallus longirostris*
COGA	Common gallinule	*Gallinula chloropus*
COGR	Common grackle	*Quiscalus quiscula*
CONI	Common nighthawk	*Chordeiles minor*
COSN	Common snipe	*Capella gallinago*
DCCO	Double-crested cormorant	*Phalacrocorax auritus*
DOWI[a]	Dowitcher	*Limnodromus* sp.
DUNL[a]	Dunlin	*Calidris alpina*
EABL	Eastern bluebird	*Siaila sialis*
EAKI	Eastern kingbird	*Tyrannus tyrannus*
EAME	Eastern meadowlark	*Sturnella magna*
FOTE	Forster's tern	*Sterna forsteri*
GLIB	Glossy ibis	*Plegadis falcinellus*
GBHE	Great blue heron	*Ardea herodias*
GREG	Great egret	*Casmerodius albus*
GHOW[b]	Great horned owl	*Bubo virginianus*
GRHE	Green heron	*Butorides striatus*
HEGU	Herring gull	*Larus argentatus*
HOME	Hooded merganser	*Lophodytes cucullatus*
KILL	Killdeer	*Charadrius vociferus*
LAGU	Laughing gull	*Larus atricilla*
LEBI	Least bittern	*Ixobrychus exilis*
LESA	Least sandpiper	*Calidris minutilla*

Table 2. (Continued, p. 3)

Code	Vernacular	Scientific name
LETE	Least tern	*Sterna albifrons*
LESC	Lesser scaup	*Athaya affinis*
LBHE[a]	Little blue heron	*Florida caerulea*
LOSH	Loggerhead shrike	*Lanius ludovicianus*
LOHE	Louisiana heron	*Hydranassa tricolor*
MODO	Mourning dove	*Zenaida macroura*
NOBO	Northern bobwhite	*Colinus virginianus*
NOCA	Northern cardinal	*Cardinalis cardinalis*
NOMO	Northern mockingbird	*Mimus polyglottos*
NOSH	Northern shoveler	*Anas clypeata*
OROR	Orchard oriole	*Icterus spurius*
OSPR	Osprey	*Pandion haliaetus*
PABU	Painted bunting	*Passerina ciris*
PBGR	Pied-billed grebe	*Podilymbus podiceps*
PIWO	Pileated woodpecker	*Dryocopus pileatus*
RBWO	Red-bellied woodpecker	*Melanerpes carolinus*
RTHA	Red-tailed hawk	*Buteo jamaicensis*
RWBL	Red-winged blackbird	*Agelaius phoeniceus*
RBGU[a]	Ring-billed gull	*Larus delawarensis*
SAND[a]	Sanderling	*Calidris alba*
SNEG	Snowy egret	*Egretta thula*
SORA[a]	Sora rail	*Porzana carolina*

Table 2. (Continued, p. 4)

Code	Vernacular	Scientific name
SPSA	Spotted sandpiper	*Actitis macularia*
TRSW	Tree swallow	*Iridoprocne bicolor*
UNTE	Unidentified tern	*Sterna* sp.
WHIB	White ibis	*Eudocimus albus*
WILL	Willet	*Catoptrophorus semipalmatus*
WOST	Wood stork	*Mycteria americana*
YRWA	Yellow-rumped warbler	*Dendroica coronata*
YELL[a]	Yellowlegs	*Tringa* sp.

[a] Observed by Cowgill (pers. comm.) during winter 1991-1992.

[b] Identified acoustically. All other birds were identified visually.

Table 3. Mammals, reptiles, and amphibians observed on the Ocean Course during this study.*

Species	Scientific name
Mammals	
Bobcat	*Lynx rufus*
Eastern cottontail	*Sylviligus floridanus*
Eastern gray squirrel	*Sciurus carolinensis*
Eastern mole	*Scalopus aquaticus*
Gray fox	*Urocyon cinereoargentatus*
Hispid cotton rat	*Sigmodon hispidus*
Marsh rabbit	*Sylviligus palustris*
Mexican freetail bat	*Tadarida brasiliensis*
Opossum	*Didelphis marsupialis*
Raccoon	*Procyon lotor*
Red bat	*Lasiurus borealis*
River otter	*Lutra canadensis*
Unidentified mouse	*Peromyscus* sp.
Whitetail deer	*Odocoileus virginianus*
Reptiles	
American alligator	*Alligator mississippiensis*
Banded water snake	*Nerodia fasciata fasciata*
Corn snake	*Elaphe guttata guttata*
Eastern glass lizard	*Ophisaurus ventralis*
Eastern kingsnake	*Lampropeltis getula getula*
Eastern mud turtle	*Kinosternon subrubrum subrubrum*
Green anole	*Anolis carolinensis*
Northern black racer	*Coluber constrictor constrictor*
Rat snake "intergrade"	*Elaphe obsoleta obsoleta x Elaphe obsoleta quadrivittata*
Unidentified rattlesnake	*Crotalus* sp.

Table 3. (Continued, p. 2)

Species	Scientific name
Amphibians	
Green treefrog	*Hyla cinerea*
Unidentified toad	*Bufo* sp.
Unidentified treefrog	*Hyla* sp.
Unidentified true frog	*Rana* sp.

* Includes species observed at the edge of the maritime forest bordering the course to the north and species observed in or around the brackish creek connecting Ibis and Willet ponds.

Table 4. Aquatic macroinvertebrates found in the Ocean Course Lagoons.

Order	Family	Species (Author)
Odonata	Aeshnidae	*Anax junius* (Drury)
	Coenagrionidae	*Ishnura posita* (Hagen)
Trichoptera	Leptoceridae	*Oecetis inconspicua* Complex
Coleoptera	Haliplidae	*Peltodytes dietrichi* (Young)
	Hydrophilidae	*Tropisternus* sp.
Diptera	Chironomidae	
	Culicidae	
Heteroptera	Belostomatidae	*Belostoma flumineum* (Say)
	Corixidae	*Trichocorixa* sp.
Amphipoda	Talitridae	*Hyalella azteca*
Decapoda	Portunidae	*Callinectes sapidus*
	Grapsidae	*Sesarma* sp.
	Ocypodidae	*Uca* sp.
		Ocypode quadrata

Identifications following [17] and [18].

Table 5. Fish species found in the Ocean Course Lagoons.

Species	Scientific name
Sheepshead minnow	*Cyprinodon variegatus*
Mummichog	*Fundulus heteroclitus*
Mosquitofish	*Gambusia affinis*
Sailfin molly	*Poecilia latipinna*
Inland silverside	*Menidia beryllina*
Striped mullet	*Mugil cephalus*
Carp	unknown
Fat sleeper	*Dormitator maculatus*
Southern kingfish	*Menticirrhus americanus*
Freshwater goby	*Gobionellus shufeldti*
Striped killifish	*Fundulus majalis*

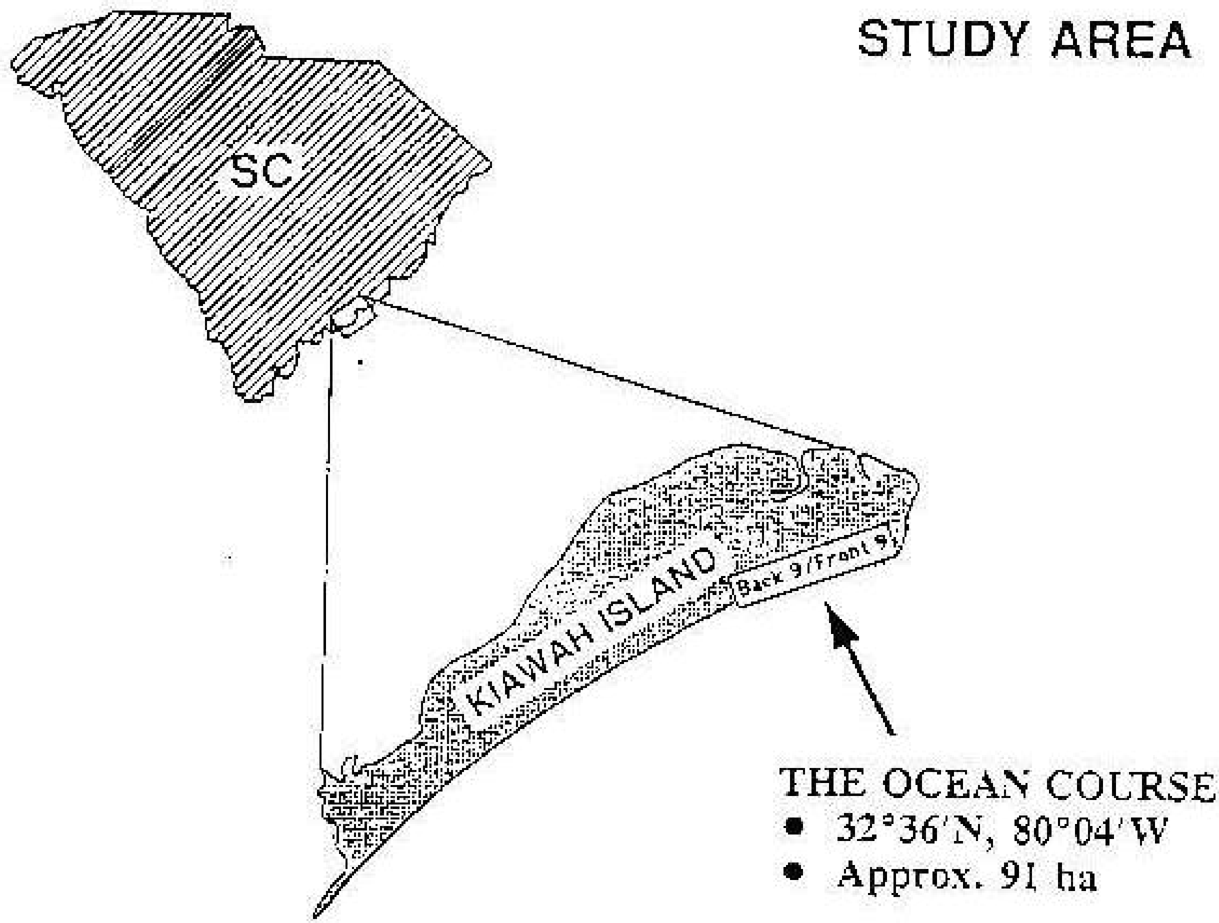

Figure 1. Location of the Ocean Course on Kiawah Island, SC.

Figure 1. Location Map

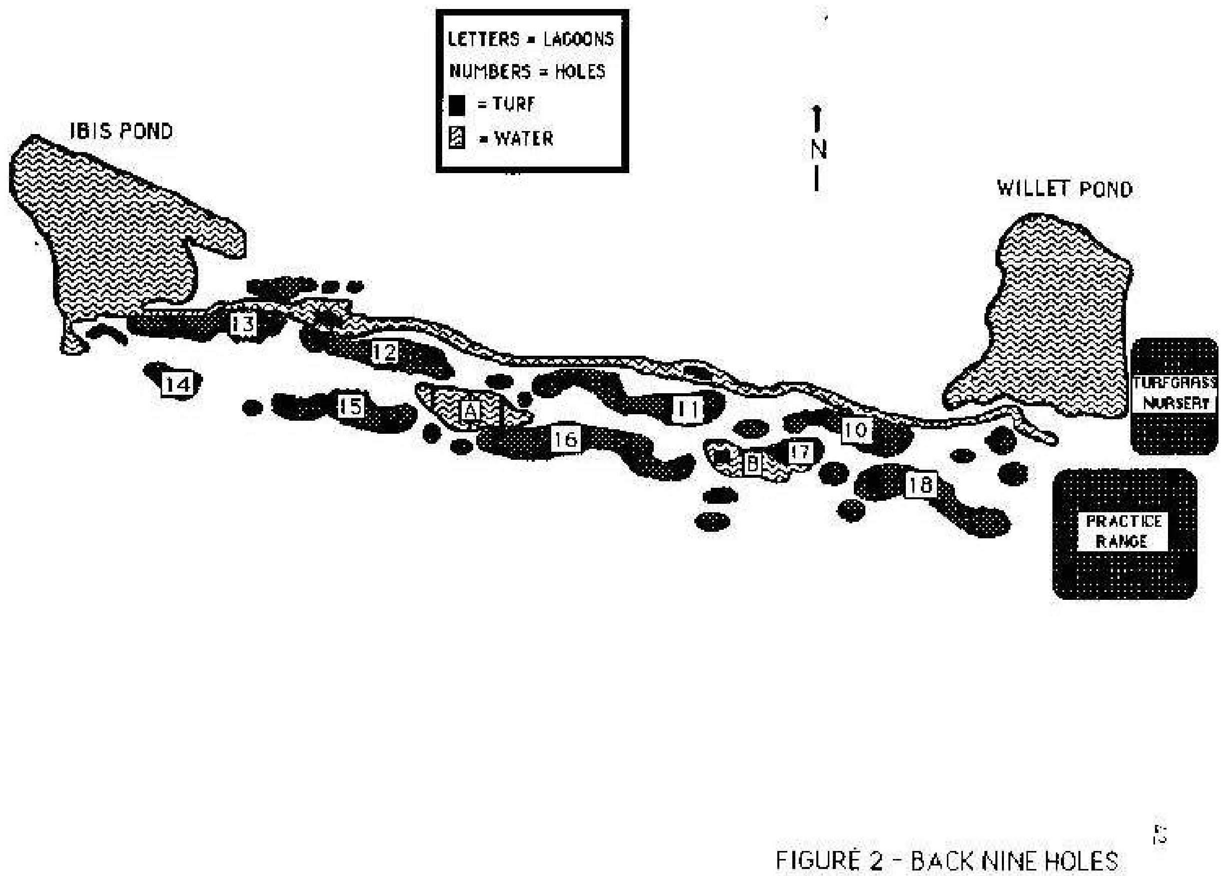

Figure 2. Back Nine Holes

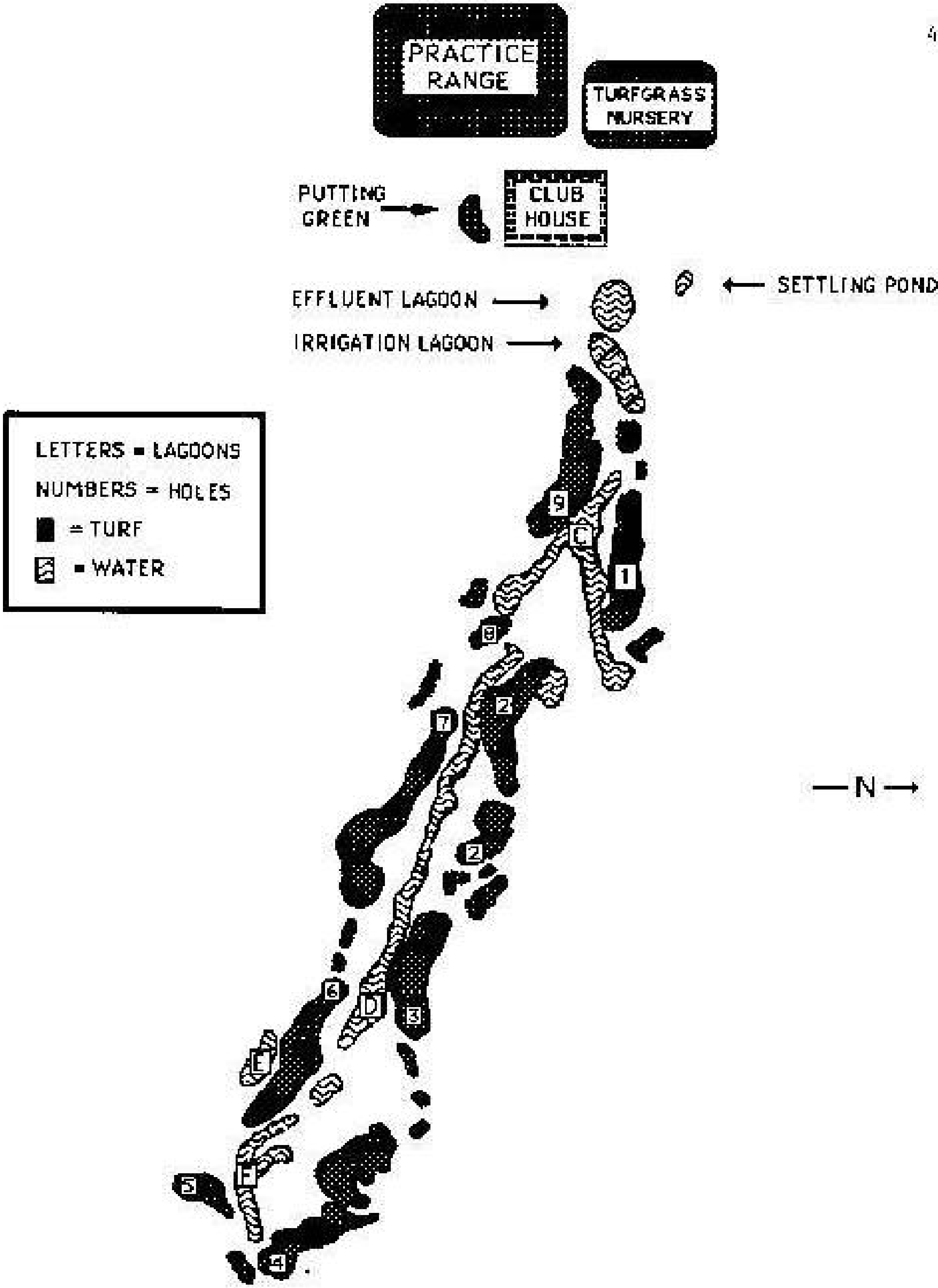

FIGURE 3 - FRONT NINE HOLES

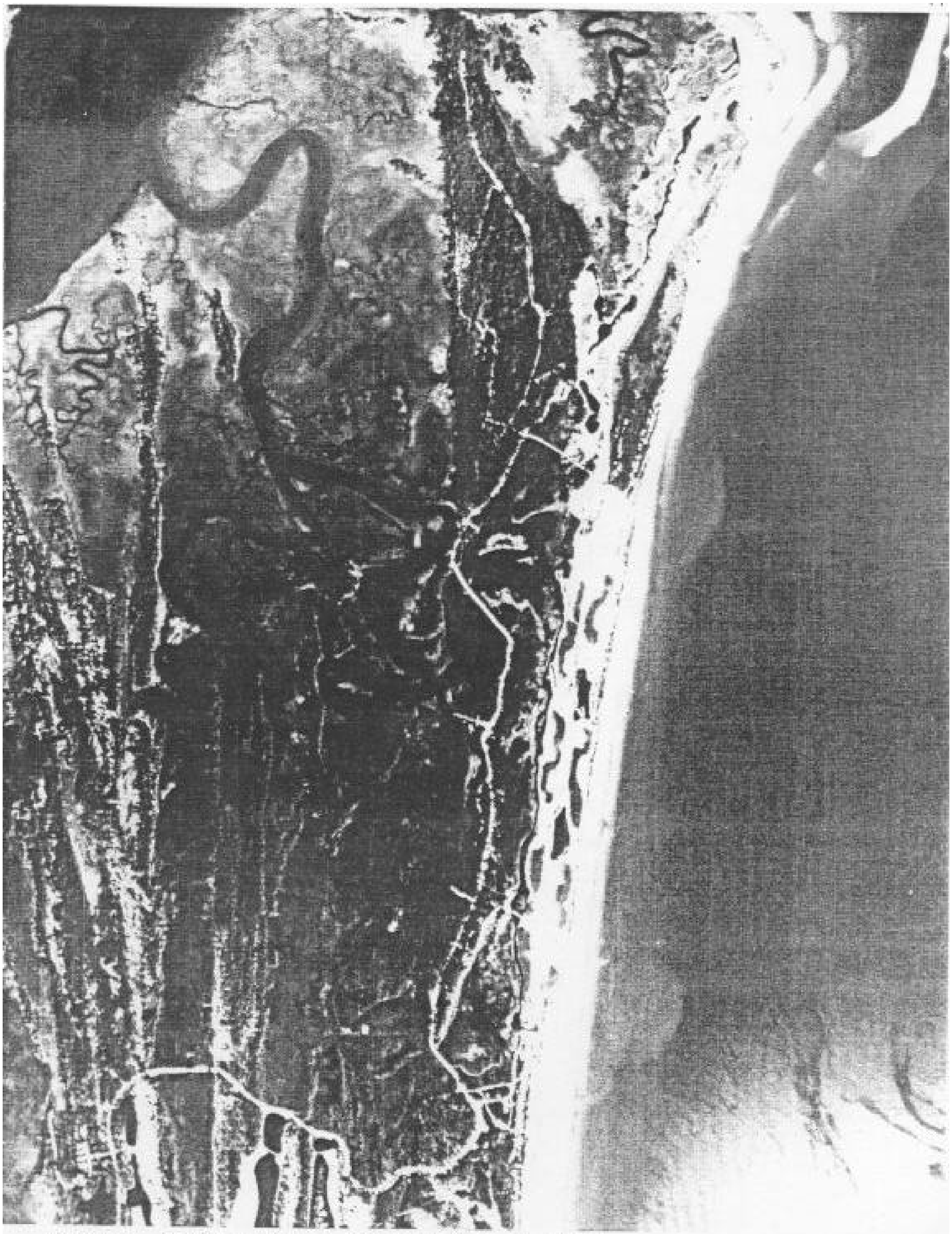

Figure 4. The Ocean Course (Kiawah Island, SC 1991)

Figure 4. The Ocean Course (Kiawah Island, SC 1991)

CHAPTER 2

AVIAN EXPOSURE TO ORGANOPHOSPHORUS AND CARBAMATE PESTICIDES ON A SOUTH CAROLINA GOLF COURSE

AVIAN EXPOSURE TO ORGANOPHOSPHORUS AND CARBAMATE PESTICIDES ON A SOUTH CAROLINA GOLF COURSE

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Abstract - A field study examined the potential for avian exposure to organophosphorus (OP) and carbamate (CA) pesticides applied to turf on a South Carolina golf course. One component of this study was to document the occurrence, distribution, and activity of birds on turfgrass. A total of 630 birds representing 17 species was counted during 240 behavioral observations from 25 May to 27 August, 1993. Birds were most commonly observed in the morning (59%), on fairways (81%), and foraging (66%) on the turf. The second component of this study was to collect blood samples and footwashes from red-winged blackbirds (*Agelaius phoeniceus*), common grackles (*Quiscalus quiscula*), and boat-tailed grackles (*Quiscalus major*) for measurement of plasma cholinesterase (ChE) activity and chemical residues, respectively. A non-significant (p<0.05) downward trend in common grackle ChE activity following pesticide applications suggests possible exposure to an anti-ChE compound. However, no pesticide residues were detected in corresponding footwash samples. An impaired laughing gull (*Larus atricilla*), discovered approximately 36 hours after an application of Turcam**7** 2.5G [active ingredient (AI),bendiocarb; 3.36 kg/ha], exhibited 87% depression of total ChE (TChE), and 50.33 ìg active ingredient were recovered in the footwash. Results of this study suggest the potential for avian exposure to pesticides on the Ocean Course, but quantification of this exposure and subsequent response is subject to further study.

Keywords - Organophosphate Carbamate Pesticides Avian field study Golf course Turf

INTRODUCTION

Organophosphorus (OP) and carbamate (CA) pesticides have essentially replaced organochlorine compounds for use in controlling agricultural and turfgrass pests [1,2] primarily because OPs and CAs exhibit a relatively short persistence in the soil, a low potential for accumulation in the food chain, and an effectiveness in controlling insects which have developed a genetic resistance to the organochlorine pesticides [3-5]. Use of these chemicals in both agricultural and urban areas, however, has been responsible for increasing avian mortality in the last several years [1,6-18]. Use of OPs and CAs on golf courses presents a particular problem because heavy applications of the pesticides are often employed to control turf insects on areas that are attractive feeding sites for grazing waterfowl and other birds [7,19]. Stone and Gradoni [10] report 16 exposure incidents from 1970 to 1985 involving 8 states and resulting in over 900 avian mortalities. One incident resulted in the death of 700 brant (*Branta bernicla*) from exposure to the OP diazinon on a New York golf course. In 1986, 85 American widgeon (*Anas americana*) died after grazing on one diazinon-treated fairway in Bellingham, Washington [16].

OP and CA pesticides act by inhibiting the enzyme cholinesterase (ChE) [20]. Cholinesterase inhibition results in an accumulation of acetylcholine at nerve synapses which disrupts normal transmission of nerve impulses. Symptoms of severe OP or CA poisoning include respiratory difficulty leading to respiratory arrest, paralysis, convulsions, coma, and death [10]. Other signs of OP/CA poisoning in birds include tremors, dyspnea, ataxia, pupil constriction, excessive salivation, and decreased heart rate [21].

Waterfowl seem to be at the greatest risk to OP and CA exposure on golf courses because many species consume the turfgrass to which pesticides are directly applied [16]. Littrell [13] reports that greens near water hazards pose the greatest threat to waterfowl because greens are often treated with OP and CA pesticides, and the 3-5 cm-high grass on the fringe of the greens is preferred by the birds for feeding. In addition to waterfowl, other species such as blackbirds (family Icteridae) and thrushes (family Turdidae) that probe the thatch layer and upper centimeter of the soil for invertebrates are also at a great risk of exposure to turf chemicals [19]. Following an application, birds can be exposed orally by ingesting dead or struggling insects [3,5,18], pesticide-impregnated particles [22,23], or contaminated water from puddles resulting from runoff [10,19]. Dermal exposure can occur by direct contact with treated turf or contaminated water, resulting in absorption of the compound through skin on legs and feet [10].

Because applications of turfgrass chemicals often coincide with avian breeding seasons when insects and birds are most abundant [3], applications in close proximity to avian breeding territories can lead to mortality of both adults and young. White et al. [1] report extensive mortality of adult and nestling laughing gulls (*Larus atricilla*) following applications of the OP, parathion, to Texas cotton fields. Adults died from ingestion of poisoned insects, while nestlings probably died from both ingestion of poisoned insects regurgitated by their parents and from starvation or exposure because their parents were killed [1].

Sublethal exposure to OP and CA pesticides may also have adverse effects on avian reproduction and behavior. Reproductive effects such as reduced egg production, reduced brood sizes, low fledgling weights, and decreased postfledgling survival may occur as a result of OP or CA exposure [24]. Aberrant behavior such as decreased nest attentiveness and decreased predator avoidance may also occur. White et al. [4] report that sublethal exposure of laughing gulls to the OP parathion may result in decreased nest attentiveness and subsequent increased susceptibility of clutches to predation or egg failure. Decreased nest attentiveness may also lead to starvation and predation of nestlings. Adult female starlings (*Sturnus vulgaris*) orally dosed with the OP dicrotophos showed 20 to 30% reduction in frequency of nestling feeding [24]. Brewer et al. [25] report brood abandonments by wood ducks (*Aix sponsa*) and teal (*Anas discors*) following an application of methyl parathion to an agricultural field. Such disturbances in avian reproduction could have significant impacts on the maintenance of population size [5].

Decreased predator avoidance has also been observed in birds exposed to anticholinesterases. Northern bobwhites (*Colinus virginianus*) dosed with methyl parathion exhibited a reduced predator escape response in both captive [26] and wild [14] scenarios. Many avian predators select prey based on movement [27], and the abnormal behavior characteristic of animals exposed to OP or CA pesticides may attract predators more readily than normal behavior [18,22]. Hunt et al. [27] report that house sparrows (*Passer domesticus*) exposed to the OP fenthion were 12 times more likely to be preyed upon by American kestrels (*Falco sparverius*) as control sparrows. Exposure to OPs and CAs may render prey species more susceptible to predation, which in turn would increase the likelihood of secondary poisoning in predators [11,21,28]. Balcomb [11] reports that red-shouldered hawks (*Buteo lineatus*) were poisoned in a Maryland cornfield after consuming prey containing high levels of the CA carbofuran.

Exposure to OPs and CAs may also elicit adverse physiological responses in birds. Pronounced hypothermia has been observed in American kestrels and northern bobwhites dosed with the OP methyl parathion [29,30]. Thermoregulation is partially under cholinergic control, and anticholinesterases such as OPs and CAs may affect temperature regulation and increase the toxic response in birds exposed to temperature stress [30]. In addition, birds exposed to OPs or CAs may also reduce their food intake, resulting in weight loss, anorexia [3,17], and sometimes death [3]. Holmes and Boag [20] report that zebra finches (*Poephila guttata*) that died after acute fenitrothion (OP) poisoning exhibited a significant weight loss and depression in body temperature. The subsequent inability to thermoregulate may have been a contributing factor in the death of severely affected individuals [20].

Approximately 80 different pesticides are currently used on South Carolina golf courses to control turfgrass pests. Approximately 10% of these are OPs and CAs [31]. Carbamates have been implicated in thousands of avian mortalities since the mid-1970s [7,22], but all reported avian exposure incidents related to pesticide use on golf courses have involved OPs only. This may be a result of limited CA usage or the fact that many exposure incidents are often not reported [21]. Nonetheless, CAs should still be considered hazardous to birds when used on golf courses.

This study was conducted on a 91 ha golf course located at the eastern-most tip of Kiawah Island, South Carolina (32E36NN, 80E04NW) (Figure 1). Completed in 1991, the Ocean Course is situated in a sensitive ecosystem and is surrounded by a variety of habitats. The "front nine" of the course (holes 1-9) (Figure 2) is bordered by maritime forest and saltwater marsh to the north and slack thicket (primarily wax myrtle [*Myrica cerifera*] and yaupon [*Ilex vomitoria*]) to the east and south. The front nine contains several small groves of live oaks (*Quercus virginiana*) and is bisected by a narrow brackish creek that in some places widens to form a series of small ponds. The "back nine" of the course (holes 10-18) (Figure 3) is bordered to the north by a wide brackish creek that connects two large brackish ponds, Ibis and Willet Ponds. Ibis Pond borders the back nine to the west, and ocean beach and dunes border to the south. Very few trees are located on the back nine. Instead, each hole is surrounded by dunes. Two large brackish ponds are located in the center of the back nine; one located between #12 and #16 fairways and the other located adjacent to #17 green. The clubhouse, putting green, practice range, and turfgrass nursery are located in the middle of the course and separate the front nine from the back nine.

Since its construction, the Ocean Course has employed the use of OP and CA pesticides to control mole crickets (*Scapteriscus* spp.) and other turf grass pests. The OPs Orthene**7** (O,S-dimethyl-acetyl-phosphoramidothioate; common name acephate), and Nemacur**7** (Ethyl-methyl-4-methylthio-phenyl-1-methylethyl phosphoramidate; common name fenamiphos) and the CA Turcam**7** (2,3-isopropylidenedioxy-phenyl methylcarbamate; common name bendiocarb) are the three pesticides that were used on the course during the 1993 field season. Toxicities of these compounds to various avian species are shown in Table 1.

Although numerous avian mortalities have been attributed to OP and CA use on golf courses, few field studies have been conducted to examine the hazard presented by these chemicals to free-ranging birds on golf courses under normal turfgrass management practices. The objectives of this study were:

(1) to characterize the potential of avian exposure to OP and CA pesticides on the study site by monitoring avian occurrence and behavior on turfgrass,

(2) to quantify exposure of selected avian species (red-winged blackbirds [*Agelaius phoeniceus*], common grackles [*Quiscalus quiscula*], boat-tailed grackles [*Quiscalus major*]) to OP and CA pesticides by measuring plasma ChE activity and chemical residues in footwashes, and

(3) to determine the feasibility of a golf course monitoring program to assess potential exposure and impacts of OP and CA pesticides to avian species under current turfgrass management practices [31].

METHODS

Golf Course Terminology

In the writing of this report, we assume the reader has a general knowledge of the game of golf, including the terminology given to the different areas of a golf course. For the purposes of this study, particularly behavioral and incidental observations, we adapted the following definitions from Moul and Elliott [32].

1. Green - the area of turf immediately surrounding the cup. A "normal" green averages 560 m^2 (range from 450-700 m^2). While the total turf area of the green is about 2% that of a golf course, greens probably receive as much traffic and maintenance as the rest of the course combined. The turf on the green is usually distinguished from other turf areas by being cut at 4.8 - 6.4 mm.

2. Collar (fringe) - a 0.9 - 1.5 m wide band of turfgrass around each green with the turf cut at 10.0 - 18.0 mm. For the purposes of this study, the collar was considered to be part of the green.

3. Approach (apron) - an area of turf extending a minimum of 15 m in all directions from a green. The turf of the approach is cut between 13.0 - 25.0 mm. During behavioral and incidental observations conducted in this study, an approach was considered to be part of the fairway.

4. Tee (tee box) - the turf surface where a golfer makes a first stroke of play on each hole. Next to the green, tees receive the highest traffic and maintenance. Turf of tees is cut between 7.6-20.0 mm. There are between one and four tee areas per hole on each course, the most common being three. The area of the tee on each hole ranges from 30-60 m^2.

5. Fairway - the turfed area of play between the tee and the green. Fairways on an average 18 hole course make up 20 ha or 2/5 of the course area. Fairways are usually between 32 and 55 m wide and the turf is cut at 13.0 - 30.0 mm.

6. Rough - the area surrounding each golf hole, which forms the background in which the game is played. It is not uncommon for rough to account for 1/2 of a course area. The turf of areas of rough is cut at 25.0 - 50.0 mm.

Chemical Applications

Nemacur**7** was applied in a granular formulation at a target rate of 11.2 kg active ingredient (AI)/ha (10 lbs AI/a). Turcam**7** was also applied as granules at a target rate of 3.36 kg AI/ha (3 lbs AI/a). Both Nemacur**7** and Turcam**7** were broadcasted using a Scotts Proturf**7** Professional Rotary Spreader with a helical cone. Areas treated with Nemacur**7** and Turcam**7** were irrigated immediately following application. Orthene**7** was applied as a liquid at the target rate of 4.2 kg AI/ha (3.75 lbs AI/a). The method of application was chemigation, a process in which the chemical is applied through the golf course irrigation system. Chemigation of Orthene**7** occurred at night, and Nemacur**7** and Turcam**7** were applied in the late afternoon when golfers were no longer present on the course. Application dates and conditions are listed in Table 2.

Wildlife Occurrence

Numerous wildlife species inhabit the Ocean Course area and were commonly seen throughout the duration of the study. We recorded all species of birds (Table 3), mammals, reptiles, and amphibians (Table 4) observed on the site during the 1992 and 1993.

Behavioral Observations

To characterize the potential of avian exposure to OPs and CAs on the golf course turf, avian occurrence and behavior were monitored on areas to which these chemicals are commonly applied. Twenty-four sites (4 tee boxes, 4 fairways, and 4 greens on both the front nine and the back nine) were randomly selected for observational purposes. Observations were conducted during morning hours (06:00-10:00) and evening hours (17:00-21:00), and each observation was 30 minutes in duration. Each site was monitored 5 times during each of the 2 time periods for a total of 10 observations per site. Observations were conducted from 25 May to 27 August, 1993.

During an observation, the species and behavior of each bird that contacted the turf was recorded. Bird behavior was divided into 5 categories:

1. foraging (FA) - the bird actively probed into the turf/thatch layer,

2. walking (WA) - the bird stood or walked on the turf,

3. loafing (LO) - the bird lay on its ventral side on the turf,

4. preening (PR) - the bird preened its feathers while on the turf,

5. drinking (DR) - the bird drank from a puddle on the turf.

Walking and loafing on OP/CA-treated turfgrass can result in dermal exposure, while foraging, preening, and drinking can lead to both dermal and oral exposure. The predominant behavior of each bird observed on turfgrass was recorded. Attempts were made to avoid counting an individual more than once during an observation. Researchers monitored sites using binoculars and attempted to be as inconspicuous as possible. Because of the open topography of the golf course, however, researcher concealment was limited, and some impact of researcher presence on avian activity most likely occurred.

Incidental Observations

Birds observed on turfgrass at times other than during behavioral observations were noted, and their behaviors were recorded. Behavior of birds observed incidentally was categorized and recorded in the same manner as in behavioral observations. We attempted to capture any birds displaying abnormal, impaired, or moribund behavior for sample collection and subsequent chemical and enzymological evaluations. Any avian carcass found on the course was collected for analysis of brain ChE and chemical residues in the gastrointestinal (GI) tract.

Trapping of Target Species

Red-winged blackbirds, common grackles, and boat-tailed grackles were selected as target species for this study because they were successfully trapped during the 1992 field season and because they were commonly observed foraging on golf course turf. In addition, red-winged blackbirds have been shown to be very sensitive to pesticides [35], and we felt this species might provide the best indication of avian exposure to chemicals used on the site. In May 1993, 11 trap sites were selected, each in an area among trees and other vegetation that provided concealment from golfers' view. Due to the lack of trees on the back nine, the majority of the trap sites (10) were located on the front nine. Each site (approximately 1 x 1 m) was cleared and leveled using a hoe. Two weeks prior to trapping, each trap site was baited with a cracked corn-wheat mix twice daily to attract target species. All bait was analyzed for chemical residues prior to use. Trapping began on 14 June and ended on 6 August. One funnel trap (approximately 1 x 1 x 0.2 m) was placed on each trap site, and trapping occurred twice daily. Traps were set and baited in the morning between 06:00 and 06:20 and closed at approximately 11:30. Traps were reopened between 16:30 and 17:00 and closed at approximately 20:30. Traps were checked approximately every hour to reduce a captured bird's time in the trap and to minimize heat stress. Traps were not set during inclement weather, and all nontarget species were released upon capture. All target species captured were weighed and marked with U.S. Fish and Wildlife Service aluminum leg bands.

Radio-tagging And Telemetric Monitoring Of Target Species

In addition to the Ocean Course, three other golf courses are located on Kiawah Island that employ the use of OP and CA pesticides to control turfgrass pests. To help determine the exposure potential of birds studied on the Ocean Course to OPs and CAs on other areas of the island, radio telemetry was employed to track the movement of target species on the Ocean Course. Twenty-three birds (8 red-winged blackbirds, 8 boat-tailed grackles, and 7 common grackles) were radio-tagged and monitored using directional hand-held telemetry equipment. Transmitters (Custom Telemetry, Watkinsville, GA) (range = approximately 0.8 km) weighed 1.2 to 1.9 grams and were equipped with a two-loop harness as described by Rappole and Tipton [36]. Loops were composed of elastic cotton thread and were adjusted in the field to fit each bird. One loop fit over each leg and was pulled up as far as possible on the proximal end of the thigh so that the transmitter rested over the feathers of the synsacrum.

Birds were tracked from 23 July through 5 August during both morning (06:00-12:00) and evening (17:00-21:00) hours. Observers drove or walked to different areas on the course and monitored the frequency of each radio-tagged bird. The status of each bird was designated as either "visual" (bird actually seen), "moving" (signal only, but change in direction of signal indicates movement), "signal" (signal only, no movement), or "off-site" (no signal). No attempts were made to track birds beyond the general Ocean Course area.

Bluebird Nest Boxes

Eastern bluebirds (*Sialia sialis*) may be attracted to dead or dying insects on golf course turf following pesticide applications [3]. Exposure to OPs and CAs directly or through consumption of contaminated food can result in ChE inhibition in both adult and nestling bluebirds [37]. In February

1992, 27 bluebird nest boxes were placed on the golf course to attract bluebirds and encourage nesting activity. In order to minimize interference with golfers, nest boxes were placed close to trees and wooded areas. Because very few trees are present on the back nine, only 6 nest boxes were placed there. The remaining 21 nest boxes were placed on the front nine. In February 1993, some nest boxes were moved to different locations. Nineteen nest boxes were placed on the front nine, and 8 were placed on the back nine. Each nest box was consistently monitored for bluebird nesting and reproductive success from April to August in 1992 and 1993. We recorded the number of nests, eggs, nestlings, and fledglings (feathered young leaving nest after 14 days post-hatch) from each nest box. Blood samples were collected from 3 nestlings in June, 1992 for plasma ChE analysis.

Collection of Biological Samples

Footwash Samples

Prior to and following chemical applications, footwash samples were collected from target species for analysis of chemical residues. Tarsi of each bird were washed with approximately 10 ml denatured ethanol, and the rinsate passed through a stainless steel funnel into a chemically clean nalgene jar equipped with a teflon lid. Between footwashes, the funnel was rinsed with ethanol. After every fifteen samples, funnel rinses were collected as field blanks to allow for detection of possible cross contamination in the collection method. Each sample was labeled and placed on ice in the field. Once out of the field, all samples were stored in a freezer at approximately -25EC. Samples were later shipped on dry ice to The Institute of Wildlife and Environmental Toxicology (TIWET) and stored at -20EC. No footwash samples were collected following Orthene7 applications because use of this compound occurred after the sample collection phase had ended.

Blood Samples

Blood samples from target species for ChE monitoring were collected via jugular venipuncture with heparinized sterile syringes and 27-gauge needles. Isopropyl alcohol was applied to the skin prior to venipuncture. Maximum blood volume drawn was determined as 1.6% of body weight. In the event of a recapture, a blood sample was drawn only if the previous sample collected from that individual had been drawn more than 48 hours before. All blood samples collected were transferred to microcentrifuge tubes and immediately placed on ice in the field. Once out of the field, samples were centrifuged at 2000 rpm for 25 minutes to separate plasma from blood cells. The plasma supernatant was then pipetted into a new microcentrifuge tube and stored in a freezer at approximately -25EC. Within 10 days of collection from the field, plasma samples were shipped on dry ice to TIWET and stored at -80EC.

Fecal-urate Samples

We attempted to collect fecal-urate samples from any bird captured on the site displaying abnormal, impaired, or moribund behavior. Samples were collected in chemically clean nalgene jars equipped with teflon lids and placed on ice in the field. Once out of the field, samples were stored in a freezer at approximately -25EC. The samples were then shipped to TIWET on dry ice and stored in a freezer at -20EC.

Invertebrate Samples

On 8 July, 1993, approximately 12 hours after an application of Turcam**7** 2.5G, treated areas were searched for dead or moribund invertebrates for residue analysis. Ten mole crickets were collected, placed in plastic storage freezer bags, and stored on ice in the field. Mole crickets were later stored in a freezer at approximately -25EC. The samples were then shipped to TIWET on dry ice and stored in a freezer at -20EC.

RESIDUE ANALYSIS

Sample Preparation

Footwash Samples

Each footwash sample was transferred from a nalgene jar to a volumetric tube. The jar was rinsed 3 times with methanol solvent, and the rinse was also transferred to the tube. Volume was reduced to 2 ml using nitrogen evaporation. All footwash samples were analyzed for both fenamiphos (active ingredient of the OP Nemacur**7**) and bendiocarb (active ingredient of the CA Turcam**7**).

Fecal-urate Samples

Approximately 1-2 grams of excreta were extracted with 30-40 ml of methanol for 20 minutes on an orbital shaker. The extract was decanted off through 5-10 grams of anhydrous sodium sulfate (Na_2SO_4) which was rinsed with methanol and collected in a 250 ml round bottom flask. Sample volume was reduced using a rotary evaporator. The extracts were quantitatively transferred to 2 ml volumetrics and brought to volume under nitrogen gas. Samples were then analyzed for bendiocarb as described below.

Invertebrate Samples

Each mole cricket was weighed, cut into fine pieces, and placed in a 125 ml erlenmeyer flask with 1 gram anhydrous Na_2SO_4. Residues were extracted with 35 ml pentane:ether [75:25] for 30 minutes on an orbital shaker. The extract was decanted off through 15 grams anhydrous Na_2SO_4 which was rinsed with methanol and collected in a 250 ml round bottom flask. Sample volume was reduced using a rotary evaporator. The extracts were quantitatively transferred to 2 ml volumetrics and brought to volume with methanol. Samples were then analyzed for bendiocarb as described below.

Residue Determination

Fenamiphos concentrations were determined by a Hewlett Packard 5890 Series II gas chromatograph with a DB-1 column (30 m x 0.53 mm I.D., 1.5 μm coating, J & W Scientific), flame photometric detector, and a Hewlett Packard 7673 auto sampler. The injection port and detector temperatures were set at 250°C and 300°C, respectively, and the oven temperatures were 140°C for 0.5 min raised to 200°C at 25°C/min, then raised at 10°C/min to 250°C which was held for 5 min. For the carrier gas, UHP Helium (Air Products, Allentown, PA), flow was set at 10.9 ml/min at 140°C.

Bendiocarb concentrations were determined by a Hewlett Packard 1090 HPLC equipped with a post column reaction module (PCK 5000, Pickering Laboratories), and a Hewlett Packard 1046 A fluorescence detector. A 15.0 cm C18 column with 5 μm packing was used (Pickering Laboratories) with a flow rate of 1.0 ml/min methanol/water mobile phase. A solvent gradient was used to separate the analytes of interest. The initial solvent mixture was 10:90 MeOH:water which was maintained for 0.5 min. The solvent mixture was adjusted to 50:50 over 12 min and then adjusted to 100% methanol over 0.5 min. The 100% methanol was eluted for 3 min, and the solvent ratio was returned to 10:90 over 1.5 min. The bendiocarb was derivatized with *o*-phthalaldehyde and measured by fluorescence detection using a 340 nm excitation and 455 nm emission wavelengths.

ChE Analysis

Plasma Preparation

Immediately prior to analysis, plasma samples were thawed and diluted 10 to 45-fold (depending on available volume) with ice-cold 0.5 M Trizma buffer (pH=7.4) solution made with de-ionized water and Preset Trizma crystals (Sigma Chemical Co., St. Louis, MO.). Each diluted sample was divided into two aliquots. One aliquot was used for determining levels of OP-inhibited ChE, and the other aliquot was used to determine levels of CA-inhibited ChE.

ChE Assays

Total ChE (TChE), acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) activities were determined using the methods of Ellman et al. [38] as modified by Hooper et al. [39]. Plasma dilutions were spectrophotometrically assayed in triplicate on a UVMax 96-well Kinetic Microplate Reader (Molecular Devices Corporation, Palo Alto, CA.). The substrate, acetylthiocholine iodide (AThCh, Sigma, assay concentration 4 x 10^{-4} M), the colorimetric agent 5,5′- dithiobis-2-nitrobenzoic acid (DTNB, Sigma, final concentration in plate 3.23 x 10^{-3} M), 0.05 M Tris (pH 7.4) buffer, and enzyme dilution (a total of 250 μl) were added to microplate wells. Tetraisopropylpyrophosphoramide (iso-OMPA, Sigma, assay concentration 1 x $10^{-3.5}$ M), a specific BChE inhibitor, was added to certain wells to selectively inhibit BChE and isolate AChE activity. ChE hydrolyzes AThCh to thiocholine and acetate. Thiocholine attacks DTNB, via a nucleophilic substitution, releasing thionitrobenzoic acid. Thionitrobenzoic acid is a yellow chromophore whose production can be followed as an increase in absorbance at 405 nm on the spectrophotometer. The rate at which the complex forms and the yellow color is generated represents total ChE activity (or AChE activity, for wells containing iso-OMPA), which is expressed as ìmoles AThCh hydrolyzed/min (or "units") per ml plasma. BChE activity was calculated as the difference between total ChE and AChE activity.

Determination of OP-inhibited ChE

Levels of OP-inhibited ChE activity were determined by a chemical reactivation method [40,41] in conjunction with the Ellman method described above. Each aliquot of diluted sample was further divided into three 500 ìl aliquots. One of the aliquots was placed on ice. The other two were used for the 2-PAM (pyridine-2-aldoxime, Sigma, assay concentration 1 x 10^{-4} M) reactivation assay. The therapeutic chemical 2-PAM displaces OPs bound to ChE, thereby reactivating the inhibited enzyme. One of the aliquots was spiked with 2-PAM and the other with an equal volume of distilled water. These two aliquots were incubated in a water bath at 25EC for 30 min. Following incubation, the two incubated aliquots and the aliquot on ice were assayed. An upper tailed Student's *t*-test was then used to compare mean activities from the values of samples run in triplicate to determine a significant increase in the 2-PAM incubated samples. Samples found to have a significant increase in activity of at least 5% after 2-PAM incubation were considered to be exposed to an OP.

Determination of CA-inhibited ChE

Levels of CA-inhibited ChE were determined using a dilution technique for promoting the reversibility of the CA-enzyme bond [42,43] in conjunction with the method described previously ("ChE assays"). A portion of each aliquot of diluted sample was assayed immediately following dilution. The remainder of each aliquot was incubated in water at 37EC for 4 hours and then assayed again. Initial values were compared to post-incubation values using the same Student's t-test mentioned under "Determination of OP-inhibited ChE".

RESULTS

Behavioral Observations

Two hundred and forty behavioral observations were conducted from 25 May to 27 August 1993. A total of 630 birds representing 17 species were observed on the golf course turf (Table 5). Laughing gulls, red-winged blackbirds, northern mockingbirds, common grackles, American crows, loggerhead shrikes, boat-tailed grackles, and killdeer were the top 8 species most commonly observed and comprised 96% of all birds counted. Laughing gulls were the most predominant birds recorded during observations, but red-winged blackbirds were observed more frequently (Table 5, Figure 4). Most birds counted were observed during morning hours (59%), and of the top 8 species only red-winged blackbirds and American crows were observed more often during evening hours (Table 6, Figure 5). Most birds were observed on the back nine of the course (59%), although 4 of the top 8 species (northern mockingbirds, common grackles, loggerhead shrikes, boat-tailed grackles) were observed more commonly on the front nine (Table 7, Figure 6). Birds were most commonly observed on fairways (81%), followed by greens (13%) and tee boxes (6%) (Table 8, Figure 7). Most birds foraged (66%) and walked (28%) on the turf during observations (Table 9, Figure 8), and laughing gulls were involved in 95% of the preening, loafing, and drinking observed.

Incidental Observations

Mortality and Morbidity Findings

On 23 January, 1993, an unidentified tern (*Sterna* sp.) was found dead in a bunker (also called a "sand trap") adjacent to #15 green. The bird was collected and stored at approximately -25EC, but was later inadvertently thrown away. The discovery of the dead tern did not coincide with a chemical application. No other avian carcasses were found on the course.

On 9 July 1993 at 08:30, an adult laughing gull (sex not determined) was discovered lying on its ventral side approximately 1 m from the edge of the turfgrass nursery. The nursery had been treated with the Turcam7 (2.5% bendiocarb) on the afternoon of 7 July (approximately 36 hours earlier). When approached, the bird made no attempt to walk or fly away. Upon closer examination, the bird exhibited some movement of its head and neck but seemed otherwise ataxic. The bird was salivating profusely and was respiring with rapid pants. The bird was placed in the shade on a towel inside a researcher's vehicle. A blood sample and footwash sample were collected. Following sample collection, fresh water and bread were made available to the bird, but the bird made no attempts to consume either. By approximately 11:25, the bird had regained its ability to stand. The bird was removed from the vehicle and placed on the ground adjacent to the practice range. Upon removal, the bird defecated on the towel, and some of the excreta were collected for chemical residue analysis. Once on the ground, the bird immediately took a few steps and flapped its wings. Eventually, the bird walked onto the practice range and joined other laughing gulls. Soon after, we could not distinguish the impaired gull from the others. We left the area and returned in approximately 15 minutes. No laughing gulls were present. We searched the adjacent vegetation and dunes for any sign of the laughing gull and found nothing. The biological samples were stored on dry ice and shipped to TIWET for analysis. The blood sample was analyzed for CA-inhibited enzyme only. Plasma TChE activity was 87% inhibited, relative to the highest reactivated activity (Figure 9). Following incubation, total plasma ChE activity increased substantially from the initial level: 325.5% after 2 hours; 507.4% after 8 hours; 656.5% after 20.5 hours; and 676.4% after 51 hours. The footwash sample contained 50.33 ìg bendiocarb, confirming that the gull had spent time on a treated area. No detectable concentration of bendiocarb was found in the fecal-urate sample.

Avian Occurrence on Turf

Nine hundred and eighty-nine birds representing 16 species were observed on the golf course turf through incidental observations (Table 10). Six species (barn swallow, brown pelican, brown thrasher, herring gull, northern bobwhite, and unidentified tern) were observed that were not observed during behavioral observations. Trends observed during incidental observations were very similar to those observed during behavioral observations. Laughing gulls, red-winged blackbirds, common grackles, northern mockingbirds, American crows, and killdeer comprised 96% of birds observed. Laughing gulls and red-winged blackbirds were again the most predominant species observed. Eighty-one percent of the birds were observed during morning hours, and 19% were observed during evening hours. Because all turfgrass areas on the course were included in incidental observations, the distribution of birds observed included two additional site types: the practice range and the turfgrass nursery. Most birds were observed on fairways (55%) followed by greens (19%), the practice range (16%), the turfgrass nursery (8%), and tee boxes (1%) (Table 11). Only laughing gulls were seen on the practice range. Most birds were observed foraging (49%) and walking (19.7%), and 97% of all preening, loafing, and drinking observed was performed by laughing gulls (Table 12).

Analysis Of Biological Samples Collected From Target Species

A total of 103 blood samples (76 from common grackles, 16 from red-winged blackbirds, and 11 from boat-tailed grackles) were collected from target species during the 1993 field season. The assay to determine levels of CA-inhibited plasma total ChE yielded the following percentages of samples exhibiting an increase in activity of at least 5%: boat-tailed grackles, 1 of 10 (10%); red-winged blackbirds, 0 of 15 (0%); and common grackles, 21 of 72 (29%). The assay to determine levels of OP-inhibited plasma total ChE resulted in no increases in activity greater than or equal to 5%. Common grackle TChE activity, relative to sampling date and pesticide application dates, is shown in Figure 10. These data represent 72 individual plasma samples collected over time. Statistical analysis of these data, using the general linear models procedure of SAS[7] (SAS Institute Inc., Cary, NC) indicate the values to be normally distributed and to have sufficiently equal variances. Differences in mean ChE activities were not statistically significant at $p<0.05$. Due to small sample sizes, statistical analyses were not performed on red-winged blackbird and boat-tailed grackle ChE activities.

Eighty-eight footwash samples were collected from target species and analyzed for fenamiphos (OP) and bendiocarb (CA) residues. No detectable concentrations of either chemical were recovered.

Telemetric Monitoring

Telemetric monitoring began on 21 July, after all 23 birds had been radio-tagged, and ended on 5 August. Signals from only 4 of the 23 birds radio-tagged were detected. Two red-winged blackbirds (frequency bands 150-7731 and 149-2518) were tracked and seen once each. These two birds were located in areas close to their respective sights of capture. The remaining 5 signals involved 2 additional birds (1 red-winged blackbird, 1 common grackle) but indicated movement only. All other birds were designated as off-site during radio-monitoring. Three radio-tagged common grackles were recaptured before all birds were tagged, but were not detected or recaptured after telemetry monitoring began.

Trapping continued for 2 weeks after telemetry monitoring ended to recover transmitters. No radio-tagged birds were recaptured.

Bluebird Nesting Activity

A total of nine nests were built during the 1992 and 1993 breeding seasons yielding 38 eggs, 25 nestlings, and 21 fledglings (Table 13). Six different nest boxes were used. Hatching success was much lower (29%) in 1993 than in 1992 (81%). Failure of eggs to hatch may be attributed to infertility or inexperience of the female as a breeder, in which case the female may fail to incubate the eggs properly [44]. Fledgling failure was only exhibited in one clutch. Four of five hatchlings disappeared from nest box #25 (1992), most likely from predation. Three blood samples collected from nestlings in 1992 were analyzed and showed no indication of OP exposure (only OPs were used on the course during the 1992 field season). Because bluebird nesting activity was relatively low, in June 1993 we decided to direct our efforts to other components of the study. However, we continued to consistently monitor each nest box for nesting and reproductive success.

Bendiocarb Residues In Mole Crickets

Bendiocarb residues were detected in six of the ten mole crickets analyzed (Table 14). Of the six mole crickets containing bendiocarb residues, the highest level was 16.89 ìg (22.02 ìg/g), and the lowest level was 0.92 ìg (1.56 ìg/g). The mean level of bendiocarb in the six contaminated crickets was 4.25 ìg (8.59 ìg/g).

DISCUSSION

Exposure Potential

Results of this study suggest the potential for avian exposure to OP and CA pesticides applied to turfgrass on the Ocean Course. Over 1600 birds representing 23 species were observed on the golf course turf during the 1993 field season. Based on behavioral observations, birds are most likely to be exposed to pesticides on mornings following chemical applications. Laughing gulls and red-winged blackbirds were implicated as the species at the greatest risk of pesticide exposure, but several other species appear to be at risk as well. Most birds foraged and walked on turfgrass, suggesting the possibility of both oral and dermal exposure. Studies report that birds commonly feed on turfgrass insects [5], and the primary route of avian exposure to anti-ChE pesticides is through ingestion of dead or struggling insects [3] and pesticide granules [23] following chemical treatment. Brewer et al. [5] observed several species of birds eating mole crickets after an application of the OP Triumph**7** on a Florida golf course. Stone and Gradoni [10] report that exposed granules are often picked up by birds foraging for seeds or grit. During the present study, on mornings following Nemacur**7** and Turcam**7** applications, dead or moribund mole crickets were prominent on treated areas and were scavenged by birds shortly after sunrise. Laughing gulls, red-winged blackbirds, northern mockingbirds, loggerhead shrikes, boat-tailed grackles, and killdeer were commonly observed feeding on treated areas, and discussions with turf workers indicated this to be a common occurrence. Recovery of chemical residues in mole crickets found on the course suggests that birds feeding on treated areas may ingest pesticide through contaminated food items.

Depending on the availability of a given OP or CA, exposure to birds may occur several days after an area is treated [6]. Contaminated mole crickets may continue to emerge from their burrows 3 to 4 days after an application [5], and chemical residues may persist on the turf surface [18,45]. Irrigation or rainfall following pesticide applications is believed to reduce the hazard to wildlife. If the soil is not readily permeable, however, puddles may form, and animals may be exposed by drinking or walking in contaminated water [5,10]. Cowles et al. [45] report that bendiocarb and chlorpyrifos applied to Ocean Course turf are retained in the turf/thatch layer. While this reduces the migration of these chemicals through the soil column thereby minimizing the risk of groundwater contamination, retention of these chemicals in the turf/thatch layer increases the potential exposure to birds. Multiple exposures may result in cumulative toxicity when repeated exposures continue to decrease ChE activity before complete recovery from earlier depressions [46]. The impaired laughing gull discovered during this study was found approximately 36 hours after an application of Turcam**7**. The bird was located on the edge of a treated area where laughing gulls had been observed foraging earlier that morning, and Turcam**7** granules were still visible on the turf surface. The condition of the bird and subsequent analysis of plasma and footwash samples indicated exposure to Turcam**7**. Dermal exposure was confirmed by recovery of 50.33 ìg bendiocarb in the footwash sample. Oral exposure could not be confirmed, but based on previous foraging exhibited by laughing gulls on the course, we suspect that the bird may have ingested contaminated food items. No bendiocarb was recovered in the fecal-urate sample, but it is possible that the parent compound may have been broken down into a more readily excretable metabolite via base hydrolysis (Weisskopf, pers. comm.), and a method for the detection of CA metabolites in excreta currently does not exist. Regardless of the laughing gull's recovery, similar exposures and subsequent incapacitation may render birds virtually defenseless against predators.

Exposure and Resulting Effects

Although results suggest the potential of avian exposure to turfgrass pesticides on the Ocean Course, there is little indication that substantial exposure actually occurs. In a separate golf course study, Kendall et al. [18] observed several avian species (including gulls, red-winged blackbirds, and American crows) feeding on areas recently treated with diazinon. The birds appeared to be feeding on intoxicated invertebrates, but no mortality or adverse behavior was observed. In the present study, although bendiocarb residues were recovered in mole crickets, the amount of chemical recovered in a given cricket was such that the number of crickets a bird would have to consume to reach a lethal dose makes the possibility of severe acute toxicity unlikely. Based on the highest level of bendiocarb found in a mole cricket (0.017 mg) and the average weights of birds trapped, Table 15 shows the number of mole crickets target species of this study would be required to ingest to reach a lethal dose of bendiocarb. Here it is also assumed that no ingested chemical is metabolized until all crickets required to reach a given lethal dose are consumed, thereby maintaining a "single dose" scenario. Because no LD50 value could be found for bendiocarb in target species, numbers of mole crickets to be consumed were calculated based on hypothetical LD50 values of 0.5, 1.0, 5.0, 10.0, 15.0, and 20.0 mg/kg.

Under these assumptions, red-winged blackbirds are the most sensitive to bendiocarb, followed by common grackles and boat-tailed grackles. At an LD50 of 1.0 mg/kg, male red-wined blackbirds, common grackles, and boat-tailed grackles would have to consume 2, 3, and 5 mole crickets, respectively, to reach a lethal dose. The numbers of ingested mole crickets required to reach a lethal dose begin to become unreasonable at an LD50 of 5.0 mg/kg: 16 for red-winged blackbirds, 31 for common grackles, and 51 for boat-tailed grackles. Beyond 5.0 mg/kg, the numbers become unrealistic. Thus, based on this "worst-case" scenario, the LD50 for bendiocarb in target species must be less than 5.0 mg/kg to cause a severe acute effect. In a true field scenario, consumption of a lethal dose of a pesticide through contaminated invertebrates will depend on several factors including toxicity of the parent compound and metabolites, biodegradation of the chemical prior to consumption, concentration of chemical in each food item, number of food items available, number of food items consumed, and the species and condition of the bird. In this study, true LD50 values for bendiocarb in these target species and more intensive sampling of mole crickets following chemical application are needed to make valid inferences as to the number of contaminated invertebrates a given bird must ingest to reach a lethal dose.

Although no LD50 values were found for bendiocarb or fenamiphos in red-winged blackbirds, common grackles, or boat-tailed grackles, Balcomb et al. [23] report severe toxicity to red-winged blackbirds when fed granules containing these compounds. One granule of Nemacur**7** 15G (15% fenamiphos) killed 20% of the red-winged blackbirds tested, five granules killed 40%, and ten granules killed 60%. Five granules of Tattoo**7** 10G (10% bendiocarb) resulted in the death of 20% of the red-winged blackbirds tested, and ten granules killed 100% of the birds.

Other than the one laughing gull, no other serious effects were observed in birds during this study. However, because only two researchers were present to scan the course for incapacitated birds following applications and because exposure to OPs and CAs often renders birds more susceptible to predation [14,27,29], it is possible that more avian exposures occurred and simply went undetected. The possibility of sublethal exposure and resulting effects [4,24-27] encourages further study.

The downward trend displayed by common grackle ChE activity after chemical applications suggests possible exposure to anti-ChE pesticides (Figure 10). However, no sick or moribund grackles were found, and no pesticide residues were detected in footwashes. The plasma samples which exhibited significant ChE reactivation (n=22) suggest avian exposure to a CA pesticide, but with minimal effects. Results of the telemetry study indicate substantial movement to off-site locations by target species. Thus, if the lower ChE activities were in fact pesticide-induced, it is possible that the birds were exposed to an anti-ChE compound on another part of the island before migrating to the Ocean Course. Similarly, birds exposed to pesticides on the Ocean Course may have moved off-site where they could not be detected by researchers.

RECOMMENDATIONS FOR FUTURE RESEARCH

Additional field studies designed to examine potential exposure and impacts of OP and CA pesticides to birds on golf courses are needed. Monitoring programs will differ depending on factors such as geographic location of the course (and subsequent climatology), avian species present, and chemical use patterns. However, cooperation between researchers and golf course managers and development of feasible methods and endpoints are both essential requirements for the success of all such monitoring programs.

Golf course managers and researchers must work together in determining the hazard presented by use of OP and CA pesticides to residing avian populations. In order for researchers to adequately monitor golf courses, course managers should provide researchers with the following:

- unlimited, though reasonable access to the golf course,
- access to pesticide application records,
- earliest possible notification of all upcoming pesticide applications,
- permission to reasonably collect all necessary environmental samples (turf, soil, water, etc.), and
- notification of any debilitated or dead wildlife observed on the course.

It is crucial that researchers be granted unlimited access to the golf course being studied. Access during early morning and evening hours is essential for trapping, mist-netting, conducting avian censuses and behavioral observations, carcass searching, and collecting biological and environmental samples following pesticide applications. If the research team consists of only a few people, as was the case in the present study, access to or permission to use a vehicle is also important. Earliest possible notification of all pesticide applications is also crucial so that researchers may efficiently organize and conduct pre- and post-application sampling. Any debilitated or dead wildlife (especially birds) observed on the course by golf course personnel should be reported to researchers so that assessment of the animal's condition can be made and pertinent samples may be quickly collected. Researchers should cooperate with golf course managers by minimizing interference of normal golf course activities (golfers' play, course maintenance work) by research activities. Researchers should also keep the course manager informed of all significant findings.

In addition to working with golf course managers, researchers must also develop feasible methods and endpoints that will allow them to monitor golf courses effectively. The feasibility of methods and endpoints will depend on the funding, personnel, and time available to conduct a given study. Therefore, given these limited resources, researchers must carefully select methods and endpoints that will provide the most useful data.

The present study suggests a potential for avian exposure to OP and CA pesticides on the Ocean Course, but no serious effects were confirmed. However, the laughing gull incident demonstrates the utility of plasma ChE measurements and chemical residue analysis as endpoints in detecting avian exposure to anti-ChE compounds. Suggestions for future research on the Ocean Course include designation of laughing gulls as primary target species; intensive behavioral observations, carcass searching, and invertebrate collection following pesticide applications; and large-scale telemetry studies to monitor the movement of selected birds on and off the golf course area. Trapping and mist netting large numbers of birds may not be feasible without interfering with normal golf course activities. In this respect, continued nest box monitoring can also serve as a valuable tool for assessing OP and

CA exposure to birds on the Ocean Course. Nest boxes can be placed in specific areas to avoid or minimize interference with golfers and golf course personnel. Nesting activity of bluebirds may increase substantially over time as the bluebird population on the Ocean Course increases and bluebirds become more accustomed to the nest boxes. European starlings should also be considered as a potential target species for nest box monitoring purposes. Starlings are commonly used in ecotoxicological studies [24,47,48] because they are geographically widespread, occur in large populations, readily accept nest boxes as nesting sites, and exhibit a high potential for exposure to pesticides by various routes [48]. Starlings were not originally selected as a target species for nest box monitoring in this study because none were previously observed on the course. To this point, no starlings have been observed on the Ocean Course. Placement of starling nest boxes on the course, however, may successfully attract a nesting population. Regardless of the species used, reproductive success and nestling ChE activity will be feasible and informative endpoints for future research. Collection of food items through esophageal constriction of nestlings and measurement of brain ChE in nestlings sacrificed or found dead should also be considered. Data generated by the use of these endpoints may assist golf course managers in developing strategies to minimize pesticide exposure and resulting impacts on residing avian species.

ACKNOWLEDGEMENTS

We would like to thank Catharine Williams for assisting in the field work and Jim Cowles and Kevin Johnson for directing chemical residue analyses. We thank the Landmark Land Company and the Ocean Course turf management staff for their cooperation during this study. We are grateful to the United States Golf Association (USGA), the Professional Golfer's Association (PGA) of America, and Monsanto Corporation for supporting this research.

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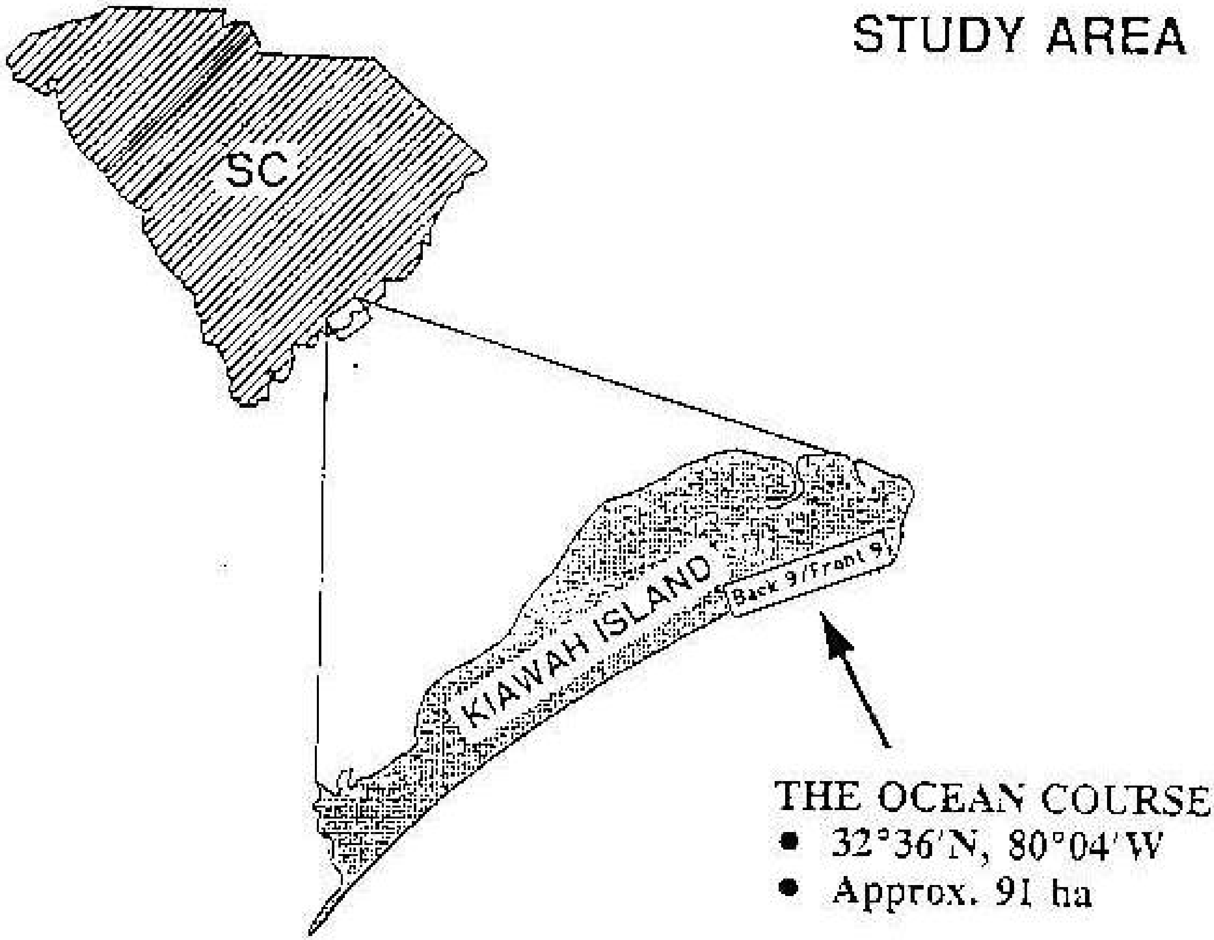

Figure 1. Location of the Ocean Course on Kiawah Island, SC.

Figure 1. Location Map

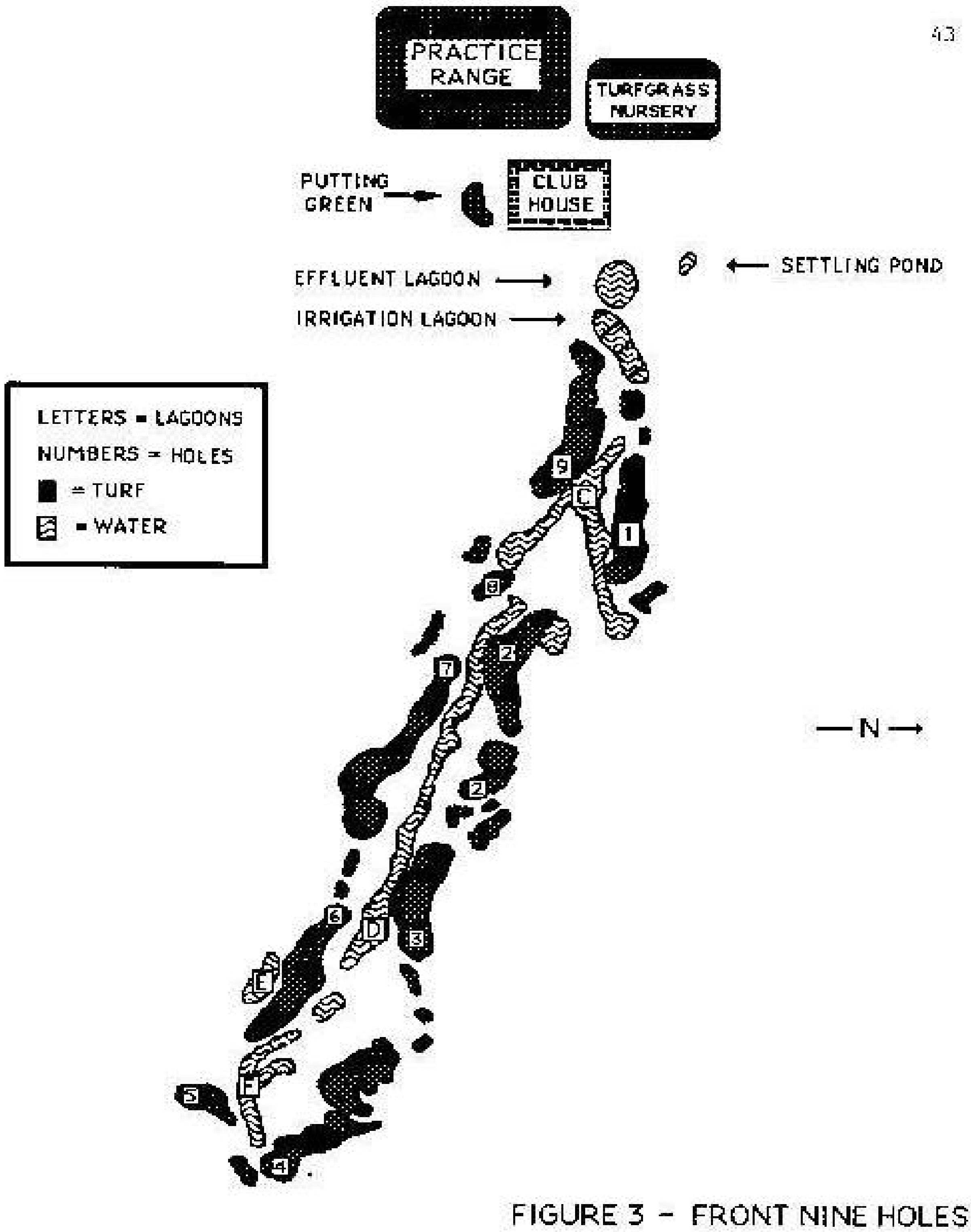

Figure 2. Front nine holes

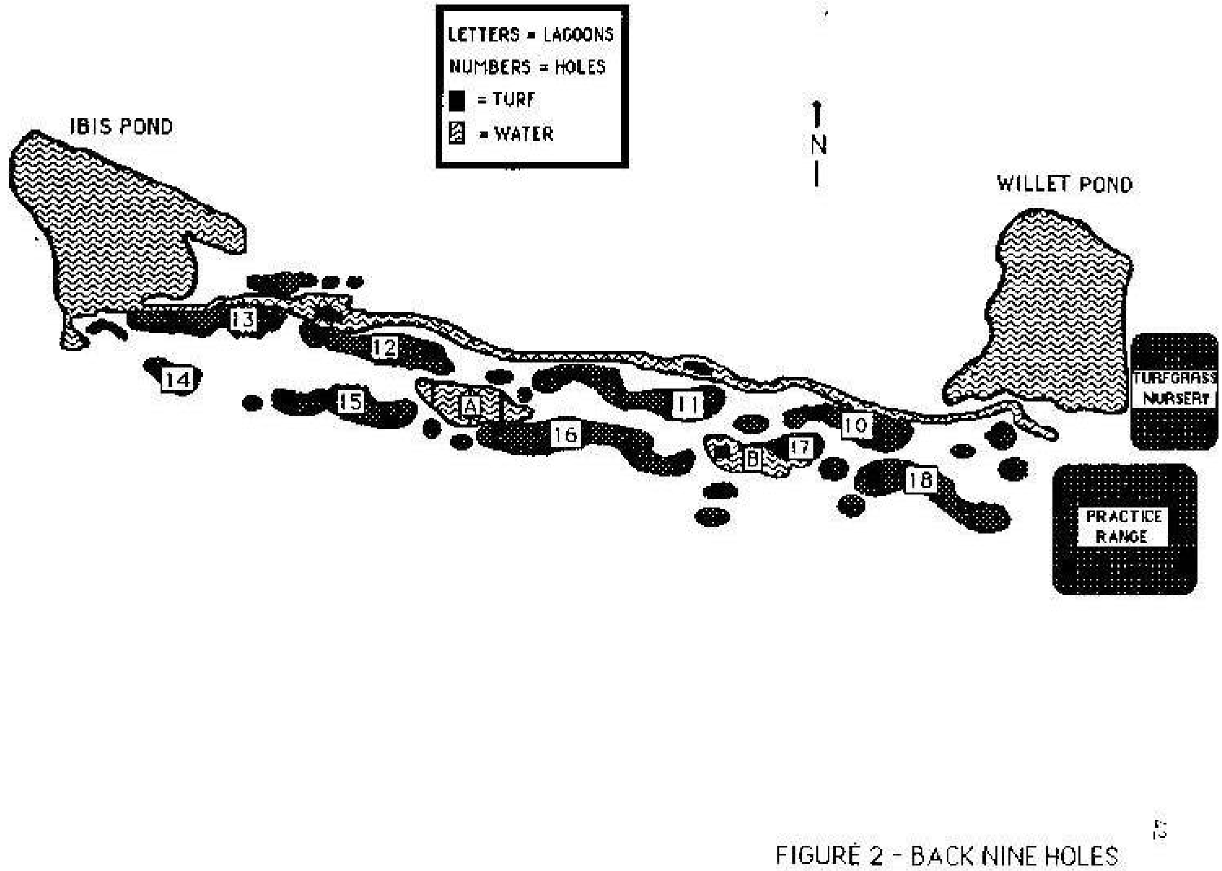

Figure 3. Back nine holes

Table 1. Summary of acute oral toxicity of the three pesticides used on the Ocean Course during 1992 and 1993 to various avian species [33].

Chemical	Test Animal	Sex	..Purity (%) or Grade	LD50 (mg/kg)
Acephate				
	Chicken	U	tech[a]	852
	Dark-eyed junco	U	75	106
	Mallard	U	tech	350
		M	93.2	234
	Ring-necked pheasant	U	tech	140
Bendiocarb				
	Northern bobwhite	M/F	tech	21
		M/F	10G[b]	33
		U	U	19[c]
	Mallard	U	U	3.1[c]
Fenamiphos				
	California quail	M	81	1.83
	Mallard	M	81	1.68
	Northern bobwhite	M/F	tech	1.0
		M/F	15G	2.4
	Ring-necked pheasant	M	81	0.5-1.0

[a]Technical

[b]Granular

[c]Reference [34]

Table 2. Information regarding insecticide applications on the Ocean Course from May-August 1993.

Date	Trade Name	Common Name	% Active Ingredient	Formulation	App. Rate (lbs/acre)	App. Method	Target Area
05/10/93*	Turcam7	Bendiocarb	2.5	granular	120	broadcast	all turf
06/17/93	Nemacur7	Fenamiphos	10	granular	100	broadcast	greens, slopes
07/07/93	Turcam7	Bendiocarb	2.5	granular	120	broadcast	approaches, tees, nursery, clubhouse
07/14/93	Turcam7	Bendiocarb	2.5	granular	120	broadcast	greens
08/05/93	Orthene7	Acephate	75	liquid	5	chemigation	front 9
08/06/93	Orthene7	Acephate	75	liquid	5	chemigation	back 9

*Approximate date

Table 3. Species codes, vernacular, and scientific names of birds observed on the Ocean Course during this study (November 1991-August 1993).

Code	Vernacular	Scientific name
AMBI	American bittern	*Botaurus lentiginosus*
AMCO	American coot	*Fulica americana*
AMCR	American crow	*Corvus brachyrhynchos*
AMOY	American oystercatcher	*Haematopus palliatus*
AMRO	American robin	*Turdus migratorius*
BASW	Barn swallow	*Hirundo rustica*
BEKI	Belted kingfisher	*Megaceryle alcyon*
BBPL[a]	Black-bellied plover	*Pluvialis squatarola*
BCNH	Black-crowned night heron	*Nycticorax nycticorax*
BNST	Black-necked Stilt	*Himantopus mexicanus*
BLSK	Black skimmer	*Rynchops niger*
BLJA[b]	Bluejay	*Cyanocitta cristata*
BWTE	Blue-winged teal	*Anas discors*
BTGR	Boat-tailed grackle	*Quiscalus major*
BOGU	Bonaparte's gull	*Larus philadelphia*
BHCO	Brown-headed cowbird	*Molothrus ater*
BRPE	Brown pelican	*Pelecanus occidentalis*
BRTH	Brown thrasher	*Toxostoma rufum*
BUFF	Bufflehead	*Bucephala albeola*
CAEG	Cattle egret	*Bubulcus ibis*
CHSP	Chipping sparrow	*Spizella passerina*
CWWI[b]	Chuck-will's-widow	*Caprimulgus carolinensis*

Table 3. (Continued, p. 2)

Code	Vernacular	Scientific name
CLRA	Clapper rail	*Rallus longirostris*
COGA	Common gallinule	*Gallinula chloropus*
COGR	Common grackle	*Quiscalus quiscula*
CONI	Common nighthawk	*Chordeiles minor*
COSN	Common snipe	*Capella gallinago*
DCCO	Double-crested cormorant	*Phalacrocorax auritus*
DOWI[a]	Dowitcher	*Limnodromus sp.*
DUNL[a]	Dunlin	*Calidris alpina*
EABL	Eastern bluebird	*Siaila sialis*
EAKI	Eastern kingbird	*Tyrannus tyrannus*
EAME	Eastern meadowlark	*Sturnella magna*
FOTE	Forster's tern	*Sterna forsteri*
GLIB	Glossy ibis	*Plegadis falcinellus*
GBHE	Great blue heron	*Ardea herodias*
GREG	Great egret	*Casmerodius albus*
GHOW[b]	Great horned owl	*Bubo virginianus*
GRHE	Green heron	*Butorides striatus*
HEGU	Herring gull	*Larus argentatus*
HOME	Hooded merganser	*Lophodytes cucullatus*
KILL	Killdeer	*Charadrius vociferus*
LAGU	Laughing gull	*Larus atricilla*
LEBI	Least bittern	*Ixobrychus exilis*
LESA	Least sandpiper	*Calidris minutilla*

Table 3. (Continued, p. 3)

Code	Vernacular	Scientific name
LETE	Least tern	*Sterna albifrons*
LESC	Lesser scaup	*Athaya affinis*
LBHE[a]	Little blue heron	*Florida caerulea*
LOSH	Loggerhead shrike	*Lanius ludovicianus*
LOHE	Louisiana heron	*Hydranassa tricolor*
MODO	Mourning dove	*Zenaida macroura*
NOBO	Northern bobwhite	*Colinus virginianus*
NOCA	Northern cardinal	*Cardinalis cardinalis*
NOMO	Northern mockingbird	*Mimus polyglottos*
NOSH	Northern shoveler	*Anas clypeata*
OROR	Orchard oriole	*Icterus spurius*
OSPR	Osprey	*Pandion haliaetus*
PABU	Painted bunting	*Passerina ciris*
PBGR	Pied-billed grebe	*Podilymbus podiceps*
PIWO	Pileated woodpecker	*Dryocopus pileatus*
RBWO	Red-bellied woodpecker	*Melanerpes carolinus*
RTHA	Red-tailed hawk	*Buteo jamaicensis*
RWBL	Red-winged blackbird	*Agelaius phoeniceus*
RBGU[a]	Ring-billed gull	*Larus delawarensis*
SAND[a]	Sanderling	*Calidris alba*
SNEG	Snowy egret	*Egretta thula*
SORA[a]	Sora rail	*Porzana carolina*

Table 3. (Continued, p. 4)

Code	Vernacular	Scientific name
SPSA	Spotted sandpiper	*Actitis macularia*
TRSW	Tree swallow	*Iridoprocne bicolor*
UNTE	Unidentified tern	*Sterna* sp.
WHIB	White ibis	*Eudocimus albus*
WILL	Willet	*Catoptrophorus semipalmatus*
WOST	Wood stork	*Mycteria americana*
YRWA	Yellow-rumped warbler	*Dendroica coronata*
YELL[a]	Yellowlegs	*Tringa* sp.

[a] Observed by Cowgill (pers. comm.) during winter 1991-1992.

[b] Identified acoustically. All other birds were identified visually.

Table 4. Mammals, reptiles, and amphibians observed on the Ocean Course during this study.*

Species	Scientific name
Mammals	
Bobcat	*Lynx rufus*
Eastern cottontail	*Sylviligus floridanus*
Eastern gray squirrel	*Sciurus carolinensis*
Eastern mole	*Scalopus aquaticus*
Gray fox	*Urocyon cinereoargentatus*
Hispid cotton rat	*Sigmodon hispidus*
Marsh rabbit	*Sylviligus palustris*
Mexican freetail bat	*Tadarida brasiliensis*
Opossum	*Didelphis marsupialis*
Raccoon	*Procyon lotor*
Red bat	*Lasiurus borealis*
River otter	*Lutra canadensis*
Unidentified mouse	*Peromyscus* sp.
Whitetail deer	*Odocoileus virginianus*
Reptiles	
American alligator	*Alligator mississippiensis*
Banded water snake	*Nerodia fasciata fasciata*
Corn snake	*Elaphe guttata guttata*
Eastern glass lizard	*Ophisaurus ventralis*
Eastern kingsnake	*Lampropeltis getula getula*
Eastern mud turtle	*Kinosternon subrubrum subrubrum*
Green anole	*Anolis carolinensis*
Northern black racer	*Coluber constrictor constrictor*
Rat snake "intergrade"	*Elaphe obsoleta obsoleta x Elaphe obsoleta quadrivittata*
Unidentified rattlesnake	*Crotalus* sp.

Table 4. (Continued, p. 2)

Species	Scientific name
Amphibians	
Green treefrog	*Hyla cinerea*
Unidentified toad	*Bufo* sp.
Unidentified treefrog	*Hyla* sp.
Unidentified true frog	*Rana* sp.

*Includes species observed at the edge of the maritime forest bordering the course to the north and species observed in or around the brackish creek connecting Ibis and Willet ponds.

Table 5. The total, frequency of detections, relative abundance, and relative frequency of birds counted on turfgrass during 240 behavioral observations from 25 May to 27 August.

	Detections			
Species	Total[a]	Frequency[b]	Relative Abundance[c]	Relative Frequency[c]
LAGU	230	35	36.5	17.3
RWBL	208	61	33.0	30.2
NOMO	47	25	7.5	12.4
COGR	32	19	5.1	9.4
AMCR	29	8	4.6	4.0
LOSH	28	18	4.4	9.0
BTGR	15	6	2.4	3.0
KILL	14	12	2.2	6.0
TRSW	7	3	1.1	1.5
WILL	7	4	1.1	2.0
COGA	6	4	1.0	2.0
EAKI	2	2	0.3	1.0
BLSK	1	1	0.2	0.5
EABL	1	1	0.2	0.5
LOHE	1	1	0.2	0.5
MODO	1	1	0.2	0.5
SPSA	1	1	0.2	0.5

[a]n=630

[b]number of behavioral observations (out of 240) in which a species was counted at least once

[c]expressed as percentage

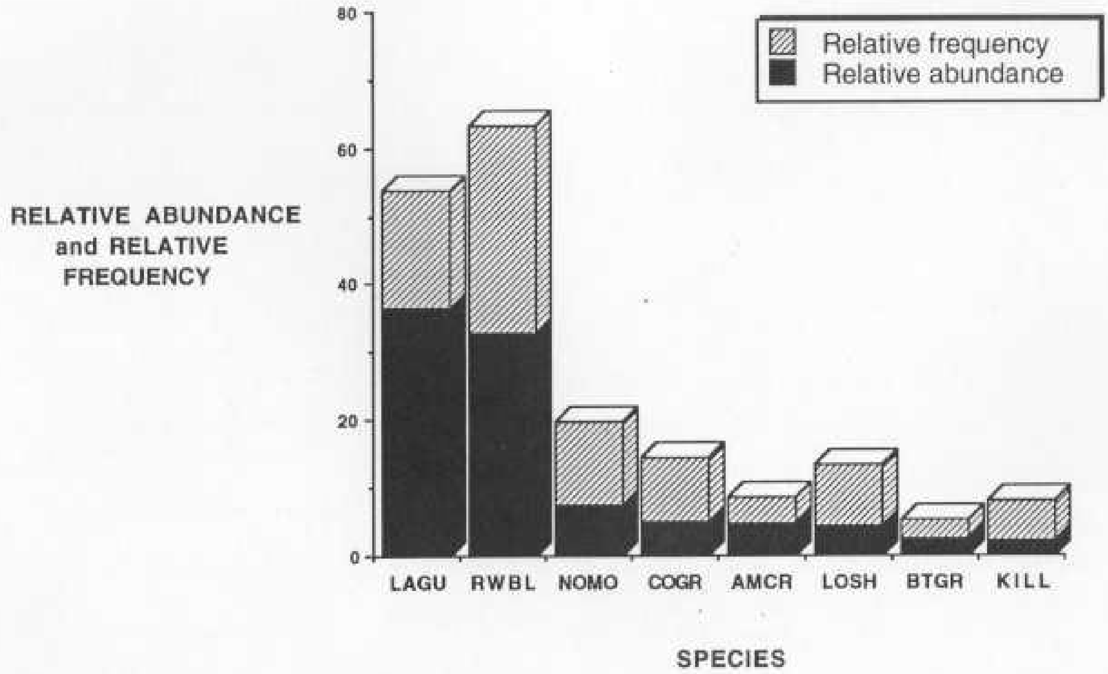

Fig. 4. Relative abundance and relative frequency of the top 8 species observed on turfgrass during behavioral observations.

Figure 4. Relative abundance and relative frequency of the top 8 species observed on turfgrass during behavioral observations.

Table 6. Morning and evening occurrence and relative abundance of birds counted on turfgrass during 240 behavioral observations from 25 May to 27 August 1993.

Species	# AM	# PM	Total	Relative Abundance (%)
LAGU	153	77	230	36.5
RWBL	96	112	208	33.0
NOMO	27	20	47	7.5
COGR	29	3	32	5.1
AMCR	10	19	29	4.6
LOSH	15	13	28	4.4
BTGR	8	7	15	2.4
KILL	11	3	14	2.2
TRSW	7	0	7	1.1
WILL	5	2	7	1.1
COGA	5	1	6	1.0
EAKI	2	0	2	0.3
BLSK	0	1	1	0.2
EABL	1	0	1	0.2
LOHE	0	1	1	0.2
MODO	1	0	1	0.2
SPSA	1	0	1	0.2
Total	371	259	630	100

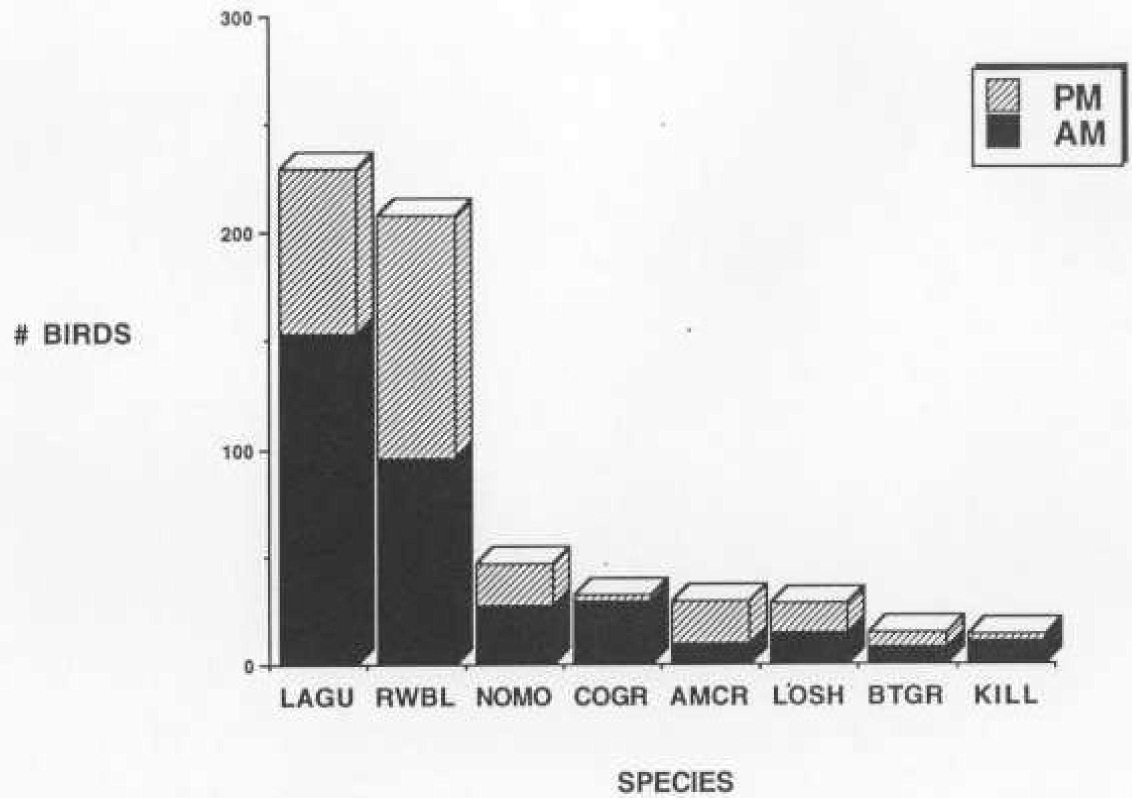

Fig. 5. Morning and evening occurrence of the top 8 species observed on turfgrass during behavioral observations.

Figure 5. Morning and evening occurrence of the top 8 species observed on turfgrass during behavioral observations.

Table 7. Morning, evening, and total occurrence of birds counted on turfgrass on the front nine and back nine of the Ocean Course during 240 behavioral observations from 25 May to 27 August 1993.

	Front Nine		Back Nine		
Species	AM	PM	AM	PM	Total
LAGU	35	6	118	71	230
RWBL	36	61	60	51	208
NOMO	23	20	4	-	47
COGR	21	3	8	-	32
AMCR	-	-	10	19	29
LOSH	11	9	4	4	28
BTGR	6	7	2	-	15
KILL	4	2	7	1	14
TRSW	5	-	2	-	7
WILL	5	2	-	-	7
COGA	-	-	5	1	6
EAKI	1	-	1	-	2
BLSK	-	-	-	1	1
EABL	1	-	-	-	1
LOHE	-	-	-	1	1
MODO	-	-	1	-	1
SPSA	1	-	-	-	1
Total (%)	58	42	60	40	-

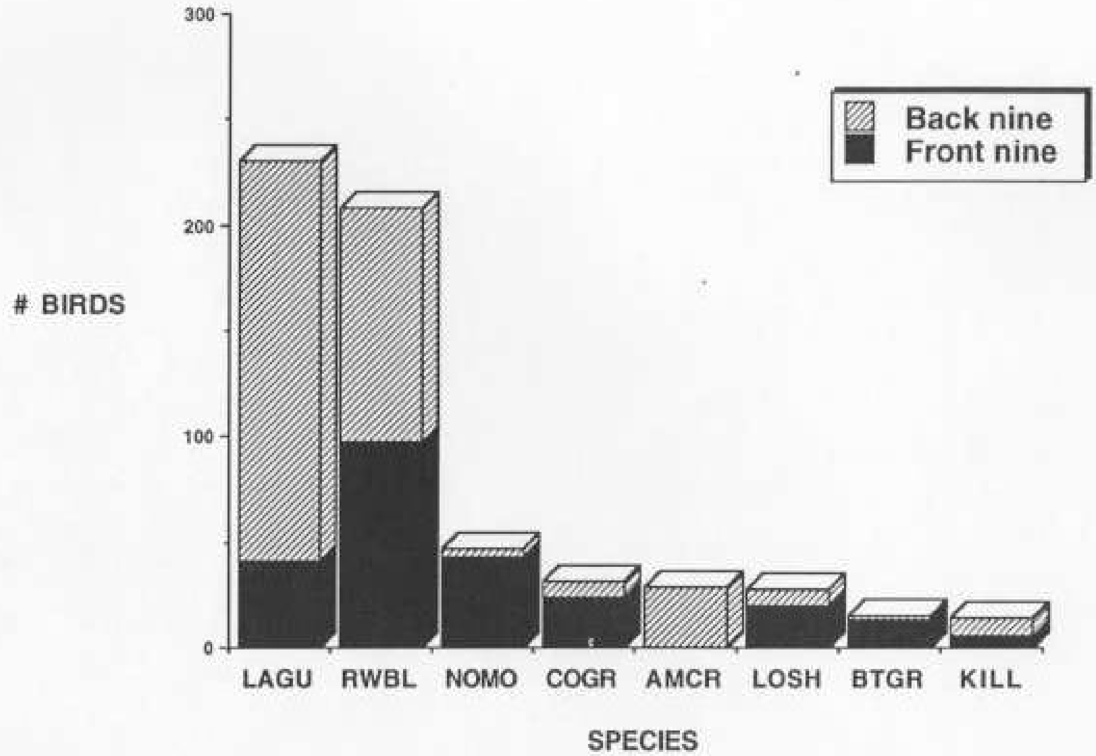

Fig. 6. Occurrence of the top 8 species on the front nine and back nine of the course during behavioral observations.

Figure 6. Occurrence of the top 8 species on the front nine and back nine of the course during behavioral observations.

Table 8. Numbers of birds counted on different turfgrass types during 240 behavioral observations from 25 May to 27 August 1993.

Species	Types of turf			Total
	Tee boxes	Fairways	Greens	
LAGU	0	222	8	230
RWBL	23	145	40	208
NOMO	2	35	10	47
AMCR	6	22	1	29
COGR	2	25	5	32
LOSH	2	18	8	28
BTGR	2	11	2	15
KILL	0	10	4	14
TRSW	0	7	0	7
WILL	0	6	1	7
COGA	1	5	0	6
EAKI	0	2	0	2
BLSK	0	1	0	1
EABL	0	1	0	1
LOHE	0	1	0	1
MODO	0	1	0	1
SPSA	0	1	0	1
Total (%)	6.0	81.0	13.0	-

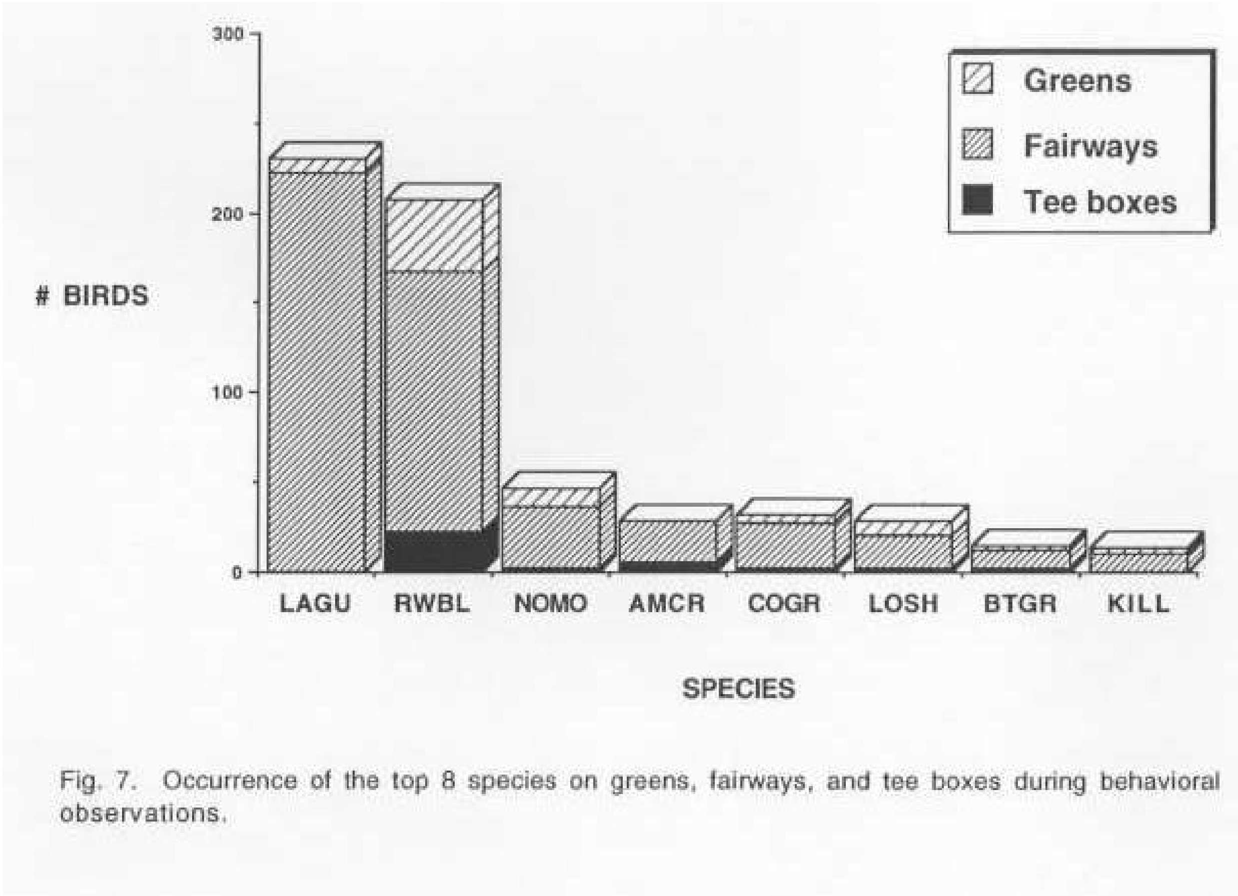

Figure 7. Occurrence of the top 8 species on greens, fairways, and tee boxes during behavioral observations.

Table 9. Behavior* of birds counted on turfgrass during 240 behavioral observations from 25 May to 27 August 1993.

	Behavior					
Species	FO	WA	PR	LO	DR	Total
LAGU	111	78	29	9	3	230
RWBL	181	27	-	-	-	208
NOMO	28	19	-	-	-	47
COGR	23	9	-	-	-	32
AMCR	25	4	-	-	-	29
LOSH	13	14	1	-	-	28
BTGR	8	7	-	-	-	15
KILL	9	5	-	-	-	14
TRSW	7	-	-	-	-	7
WILL	1	6	-	-	-	7
COGA	3	3	-	-	-	6
EAKI	2	-	-	-	-	2
BLSK	-	-	-	1	-	1
EABL	1	-	-	-	-	1
LOHE	-	1	-	-	-	1
MODO	-	1	-	-	-	1
SPSA	1	-	-	-	-	1
Total (%)	65.6	27.6	4.8	1.6	0.5	-

*Behavoir codes: FO=foraging

WA=walking
PR=preening
LO=loafing
DR=drinking

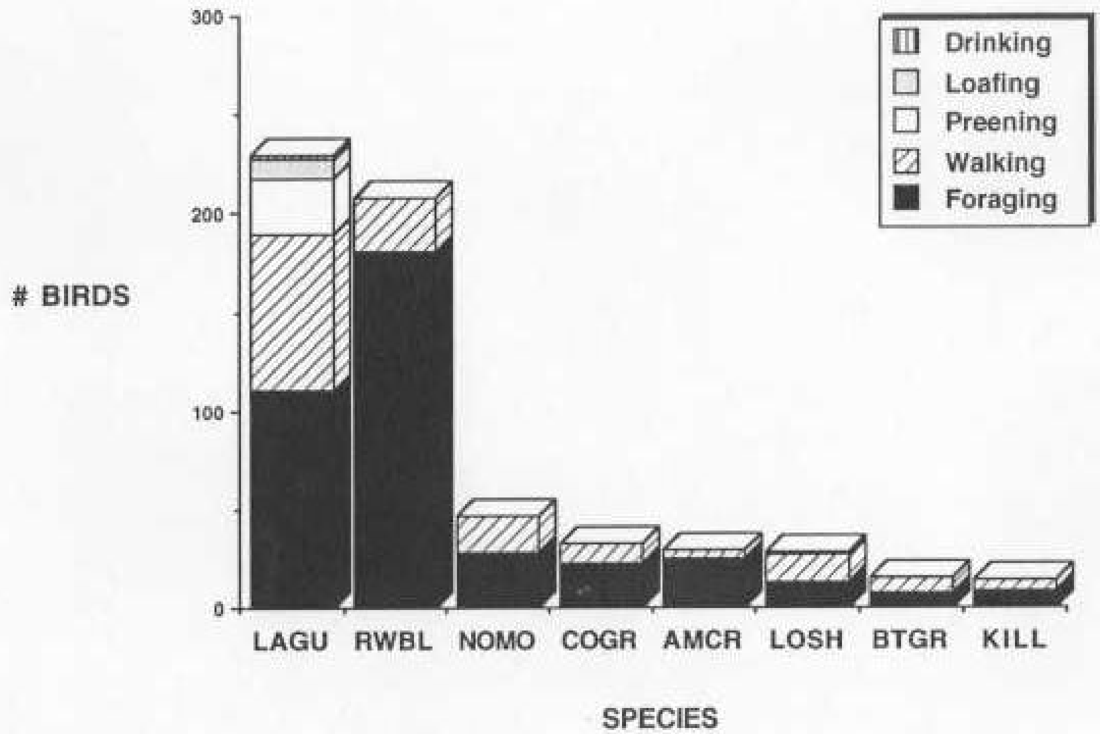

Fig. 8. Behaviors exhibited by the top 8 species observed on turfgrass during behavioral observations.

Figure 8. Behaviors exhibited by the top 8 species observed on turfgrass during behavioral observations.

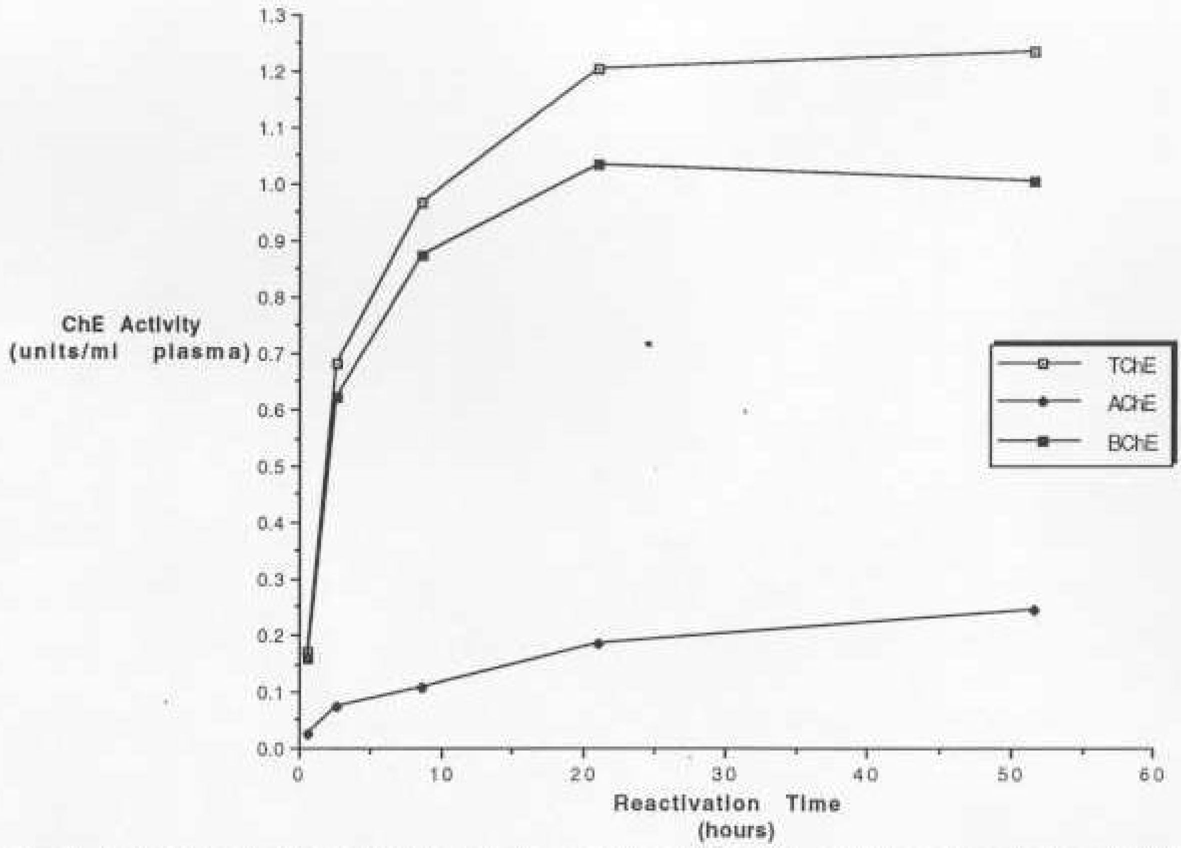

Fig. 9. Induced reactivation of laughing gull plasma ChE activity following dilution and incubation at 37°C for indicated times. Reactivation was significant at all time points following initial sample assay.

Figure 9. Reactivation of laughing gull plasma ChE activity over a period of 51 hours.

Table 10. Morning and evening occurrence and relative abundance of birds observed on turfgrass by incidental observation from May to August 1993.

Species	# AM	# PM	Total	Relative Abundance (%)
LAGU	484	80	564	57.0
RWBL	203	38	241	23.4
COGR	7	42	49	5.0
NOMO	22	10	32	3.2
AMCR	25	5	30	3.0
KILL	24	6	30	3.0
LOSH	8	8	16	1.6
BASW	8	-	8	0.8
BTGR	8	-	8	0.8
COGA	5	-	5	0.5
BRPE	-	1	1	0.1
BRTH	1	-	1	0.1
HEGU	1	-	1	0.1
NOBO	-	1	1	0.1
TRSW	1	-	1	0.1
UNTE	1	-	1	0.1
Total	798	191	989	100

Table 11. Numbers of birds counted on different turfgrass types by incidental observation from May- August 1993

	Types of turf					
Species	Tee boxes	Fairways	Greens	Practice Range	Nursery	Total
LAGU	-	357	12	154	41	564
RWBL	12	66	143	-	20	241
COGR	-	45	4	-	-	49
NOMO	1	17	14	-	-	32
AMCR	-	18	7	-	5	30
KILL	-	9	4	-	17	30
LOSH	-	14	2	-	-	16
BASW	-	8	-	-	-	8
BTGR	-	5	3	-	-	8
COGA	-	3	2	-	-	5
BRPE	-	1	-	-	-	1
BRTH	-	1	-	-	-	1
HEGU	-	1	-	-	-	1
NOBO	1	-	-	-	-	1
TRSW	-	1	-	-	-	1
UNTE	-	1	-	-	-	1
Total (%)	1.4	55.3	19.3	15.6	8.4	-

Table 12. Behavior of birds counted on turfgrass by incidental observation from May-August 1993.

	Behavior					
Species	FO	WA	PR	LO	DR	Total
LAGU	113	151	125	171	4	564
RWBL	230	9	-	2	-	241
COGR	46	2	-	-	1	49
NOMO	16	16	-	-	-	32
AMCR	28	2	-	-	-	30
KILL	27	3	-	-	-	30
LOSH	10	6	-	-	-	16
BASW	3	-	-	5	-	8
BTGR	6	2	-	-	-	8
COGA	5	-	-	-	-	5
BRPE	-	1	-	-	-	1
BTRH	-	1	-	-	-	1
HEGU	-	-	-	1	-	1
NOBO	-	1	-	-	-	1
TRSW	1	-	-	-	-	1
UNTE	-	1	-	-	-	1
Total (%)	49.0	19.7	12.6	18.1	0.5	989

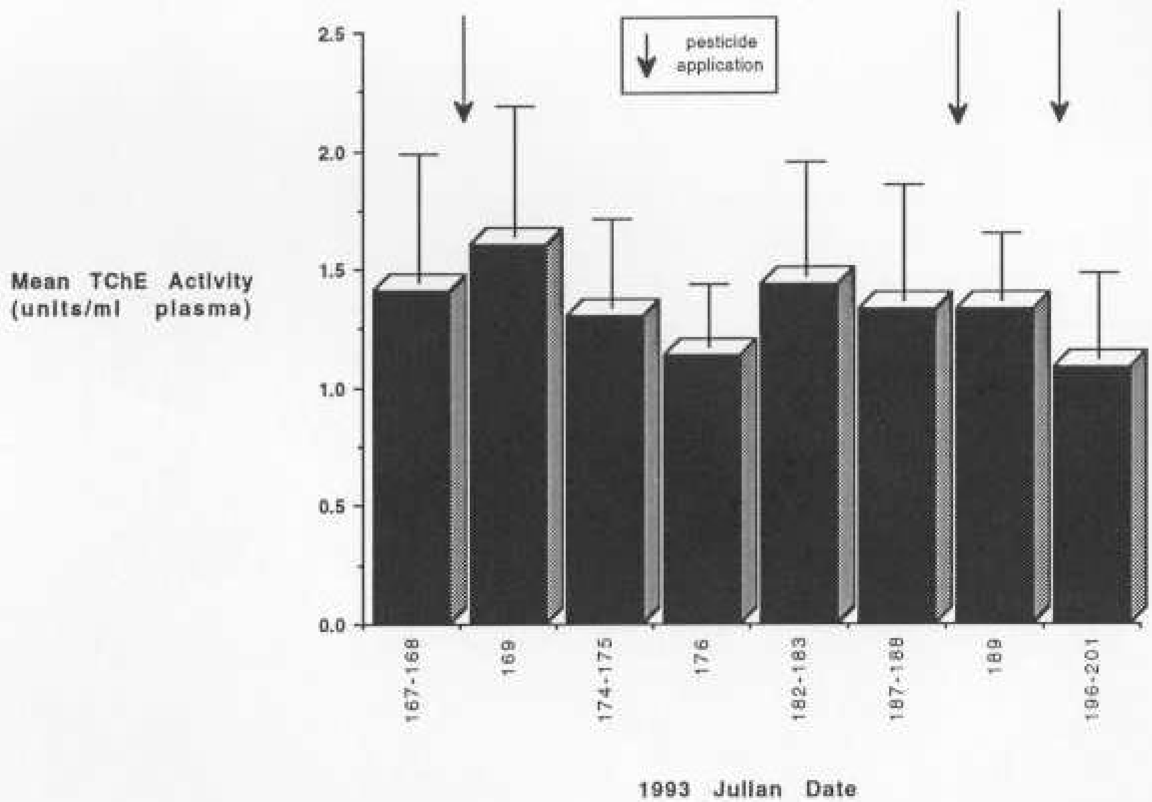

Fig. 10. Common grackle ChE activity (± S.D.) showing a decreasing pattern (not significant at $p<0.05$) after pesticide applications.

Figure 10. Common grackle ChE activity showing a decreasing pattern after pesticide applications.

Table 13. Nesting activity of eastern bluebirds in artificial nest boxes during the 1992 and 1993 breeding seasons.

Nest box #	# Eggs	# Hatched	# Fledged
1992			
6[a]	4	3	3
24	4	4	4
25[b]	5	5	1
23	4	4	4
6[a]	4	1	1
1993			
1	4	1	1
24	4	3	3
25	5	3	3
18	4	1	1
Total	38	25	21

[a]Appeared in the 1992 report as nest box #3.
[b]Appeared in the 1992 report as nest box #26.

Table 14. Sites of collection, weights, and levels of bendiocarb in mole crickets collected after an application of Turcam7 2.5G.*

Mole cricket Sample #	Site of collection	Wet weight (g)	Total bendiocarb (ìg)	Total bendiocarb (ìg/g)
5	#7 tee	0.592	0.92	1.56
6	#7 tee	0.638	1.14	1.78
7	#1 approach	0.548	2.07	3.77
8	#1 approach	0.767	16.89	22.02
9	#9 approach	0.659	3.14	4.77
10	#9 approach	0.075	1.32	17.61
		Max. level	16.89	22.02
		Min. level	0.92	1.56
		Avg. level	4.25	8.59

*Four of the ten mole crickets collected had no detectable levels of bendiocarb.

Table 15. Number of mole crickets that must be consumed by target species to reach various lethal doses.*

		# mole crickets that must be ingested to reach lethal dose	
Assumed LD50 (mg/kg)	Species	Males	Females
0.5	RWBL	2	1
	COGR	3	3
	BTGR	5	4
1.0	RWBL	3	3
	COGR	6	6
	BTGR	10	8
5.0	RWBL	16	14
	COGR	31	28
	BTGR	51	38
10.0	RWBL	33	29
	COGR	62	56
	BTGR	101	76
15.0	RWBL	49	43
	COGR	94	84
	BTGR	152	114
20.0	RWBL	66	58
	COGR	125	112
	BTGR	202	152

*Assumptions:

1. Each mole cricket contains 0.017 mg bendiocarb (highest level found in mole crickets analyzed; n=10).
2. Mean body weights (kg) of birds trapped are as follows.
   RWBL: Males = 0.056 (n=23); Females = 0.049 (n=1)
   COGR: Males = 0.106 (n=63); Females = 0.095 (n=43)
   BTGR: Males = 0.172 (n=4); Females = 0.129 (n=12)
3. No ingested chemical is metabolized until all mole crickets required to reach a given lethal dose are consumed.

CHAPTER 3

BIOCHEMICAL ASSESSMENT OF AVIAN EXPOSURE TO PESTICIDES ON A SOUTH CAROLINA COASTAL GOLF COURSE

BIOCHEMICAL ASSESSMENT OF AVIAN EXPOSURE TO PESTICIDES ON A SOUTH CAROLINA COASTAL GOLF COURSE

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Abstract C Plasma from birds on the Kiawah Island Ocean Course was evaluated for its utility in determining pesticide-induced non-target effects. Plasma cholinesterase (ChE) activity was determined and reactivated with two methods to free organophosphorus- and carbamate-inhibited ChE. Analysis of 72 common grackle (*Quiscalus quiscula*) plasma samples suggested depression (not significant at p<0.05) in ChE activity post chemical application. One incapacitated laughing gull (*Larus atricilla*) was determined to have 87% inhibition of plasma total ChE and 50 micrograms of bendiocarb (active ingredient of Turcam7) in a footwash. ChE reactivation analysis is a useful tool in determining cause of wildlife poisoning incidents, and may help golf course managers plan chemical applications.

Keywords C Organophosphorus Carbamate Avian Cholinesterase inhibition Golf course

INTRODUCTION

Many pesticides commonly used on golf courses are either organophosphorus (OP) or carbamate (CA) compounds which act as anti-cholinesterases (anti-ChEs) [1,2]. OPs and CAs inhibit cholinesterases (ChEs) vital for the proper function of cholinergic neurons. In such neurons, acetylcholine transmits nerve impulses by diffusing into the synaptic gap and causing depolarization of the post-synaptic cell. The enzyme acetylcholinesterase (AChE) catalyzes the hydrolysis of acetylcholine in the synaptic gap resulting in the vital repolarization of the post-synaptic membrane. Thus, ChE serves to regulate transmission of nerve impulses by controlling the amount of neurotransmitter, acetylcholine, present in the synapse. OP and CA insecticides characteristically have a high affinity for ChEs.

Butyrylcholinesterase (BChE) may protect the nervous system against such pesticides. However, when the plasma concentration of inhibitor is sufficient, AChE is inhibited and acetylcholine accumulates in the synapse. The post-synaptic cell remains depolarized causing paralysis of that nerve and its affected tissue, whether nervous, muscular or glandular. When enough nerves controlling vital functions are inhibited by an anti-ChE compound, the animal will become incapacitated and may die.

Though many turf pests are effectively controlled with OP and CA insecticides, concurrent poisoning of non-target species can result. Avian species may be particularly susceptible to non-target poisoning as they feed on turf or on insects containing chemical residues [1,3,4]. Fish may be exposed to contaminated runoff entering waterways during the frequent irrigation of golf course turf. The levels of free, uninhibited ChE is depressed in wildlife exposed to anti-ChE insecticides. Thus, the magnitude of OP and CA exposure can be estimated by measuring the amount of ChE inhibition.

This biochemical assessment is part of a larger study to understand effects of golf course management practices on wildlife at the Kiawah Island Ocean Course, South Carolina. Our objective was to determine whether or not avian and piscine species were measurably exposed to OP and CA insecticides during normal management practices. The purpose of this paper is to report the results of the avian plasma ChE measurements.

METHODS

Avian plasma samples (n=104) were obtained from the Ocean Course. These samples represented 76 common grackles (*Quiscalus quiscula*), 11 boat-tailed grackles (*Quiscalus major*), 16 red-winged blackbirds (*Agelaius phoeniceus*) and 1 laughing gull (*Larus atricilla*). Heparinized blood samples were centrifuged, the cellular fraction discarded, and the plasma immediately frozen at -20EC. Within 10 days, the frozen samples were transferred 5 hours on dry ice to a -80EC freezer and stored until analysis.

Immediately before analysis, samples were thawed and diluted 10- to 45-fold (depending on the available volume) with a 0.05 M Trizma buffer pH 7.4. Total ChE, AChE and BChE activities were determined in a single assay using the method of Ellman et al. [5] modified by Hooper et al. [6], for use on a UVMax kinetic microplate spectrophotometer (Molecular Devices Corp., Palo Alto, CA). The change in absorbance was converted to micromoles substrate hydrolyzed per minute per mL. The substrate for enzyme action was acetylthiocholine iodide (AThCh, assay concentration 4 X 10^{-4} M). Final assay concentration of the colorimetric agent 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) was 3.23 X 10^{-3} M. Plasma AChE activity was that remaining after samples were treated with the specific BChE inhibitor, tetraisopropylpyrophosphoramide (iso-OMPA, assay concentration 1 X $10^{-3.5}$ M). BChE activity was calculated as the difference between total ChE and AChE activity. All enzyme activities were measured in triplicate. All reagents were purchased from Sigma Chemical Company, St. Louis, MO.

Levels of OP-inhibited ChEs were determined by a chemical reactivation method [7,8] in conjunction with the modified Ellman method described above. Diluted plasma samples were divided into two aliquots and incubated 20 minutes at 25EC in the absence and presence, respectively, of pyridine-2-aldoxime methochloride (2-PAM, incubation concentration 1 X 10^{-4} M) which displaces OPs bound to ChE, thus reactivating the enzyme. After incubation, samples were assayed for total ChE, AChE and BChE activities. A one-tailed Student's *t* test was used to compare the means from the triplicate values to determine if there was a significant increase (p<0.05) in the 2-PAM-incubated aliquots.

Levels of CA-inhibited ChE were determined using a dilution technique for promoting the reversibility of the carbamate-enzyme bond [9,10] in conjunction with the modified Ellman method described above. Initial activities of total ChE, AChE and BChE were determined immediately after dilution. Then, samples were incubated at 37EC for 4 hours. Post-incubation enzyme activities were determined and compared to initial values. The same one-tailed Student's *t*-test used in the 2-PAM method was used to determine if post-incubation activities were significantly increased (p<0.05).

All enzyme activities were expressed in units/mL plasma, where one unit is defined as the activity which hydrolyzes one micromole of substrate per minute. Common grackle total ChE activities were evaluated with the general linear models procedure of SAS[7] (SAS Institute Inc., Cary, NC). Such analyses were not completed for red-winged blackbirds and boat-tailed grackles due to small sample size.

RESULTS

Common grackle total ChE activity, relative to sampling date and insecticide application dates, is shown in Table 1 and Figure 1. Individual values were normally distributed and had sufficiently equal variances. No significant differences ($p<0.05$) were found between groups.

The reactivation assay to determine levels of CA-inhibited plasma total ChE resulted in the following percentages of tested samples whose total ChE activity increased at least 5%: boat-tailed grackles, 1 of 10 (10%); red-winged blackbirds, 0 of 15 (0%); and common grackles, 21 of 73 (29%). The reactivation assay to determine levels of OP-inhibited plasma total ChE resulted in no increases greater than or equal to 5% in any samples.

Carbamate reactivation analysis of the laughing gull plasma resulted in the most substantial levels of reactivated total ChE. Following incubation, percent increases above the initial value were as follows: 325.5% after 2 hours; 507.4% after 8 hours; 656.5% after 20.5 hours; and 676.4% after 51 hours. Initial total ChE activity was, therefore, 87% inhibited relative to the highest reactivated activity (Figure 2).

DISCUSSION

Seventy-two common grackles sampled during June and July 1993 exhibited a pattern of ChE activities which suggest that this species may be responding to anti-ChE insecticide applications. Though differences in mean ChE activities were not statistically significant at $p<0.05$, a downward trend in ChE activity after each chemical application suggests the possibility of low exposure levels, encouraging further research. The individual avian samples which were significantly reactivated more than 5% suggest that those birds were exposed to CA insecticides but minimally affected.

The laughing gull plasma ChE was at least 87% inhibited, as shown by a reactivation assay designed to free CA-inhibited ChE. This gull was found paralyzed 1 m from turf which had received a carbamate application approximately 36 hours earlier. Chemical analysis of a footwash from this gull indicated the presence of 50.33 micrograms bendiocarb (active ingredient of Turcam[7]). These biological and chemical data confirm pesticide-induced incapacitation of one bird during the 1993 field season at the Kiawah Ocean Course. Such anti-ChE exposure in birds has been shown to result in decreased body weight and temperature [11,12] and increased susceptibility to predation [13,14].

Biochemical assessment of avian exposure to pesticides is an important tool in evaluating chemical use. Sampling large numbers of birds may not be feasible on many golf courses. In this study, no serious effects due to anti-ChE pesticides were found among extensively sampled species. However, the laughing gull incident demonstrates the utility of plasma ChE reactivation assays, supported by residue analysis, in determining chemical exposure during non-target poisoning incidents. Golf course managers wishing to minimize non-target pesticide effects would be well-served by biochemical assessments of wildlife mortality and morbidity incidents. Determination of the cause of such incidents, whether pesticide-related or not, will aid in planning environmentally-sensitive chemical application practices.

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Table 1. Common grackle total cholinesterase activity and number of carbamate reactivations relative to insecticide applications.

Julian Date	*N*	Carbamate Reactivations	Units/mL plasma (X"S.D.)
167-168	11	4	1.412 " 0.5372
168 (OP)	-	-	-
169	4	1	1.607 " 0.5494
174-175	8	2	1.306 " 0.3747
176	8	5	1.142 " 0.2649
182-183	18	3	1.441 " 0.4761
187-188	6	4	1.338 " 0.4838
188 (CA)	-	-	-
189	10	1	1.336 " 0.2815
195 (CA)	-	-	-
196-201	7	1	1.085 " 0.3649

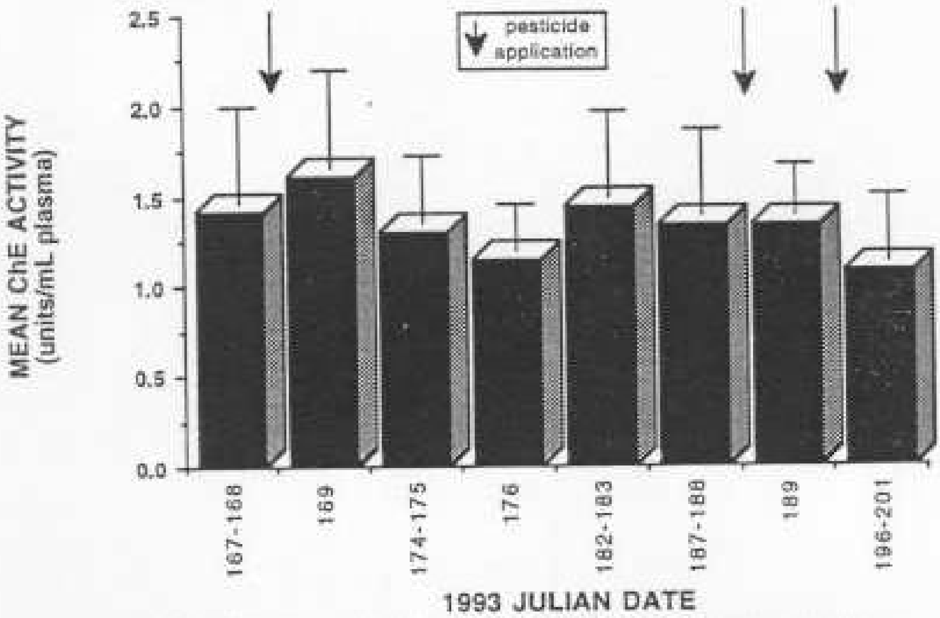

Fig. 1. ChE activity in common grackles showed a decreasing pattern (not significant at $p<0.05$) after insecticide applications.

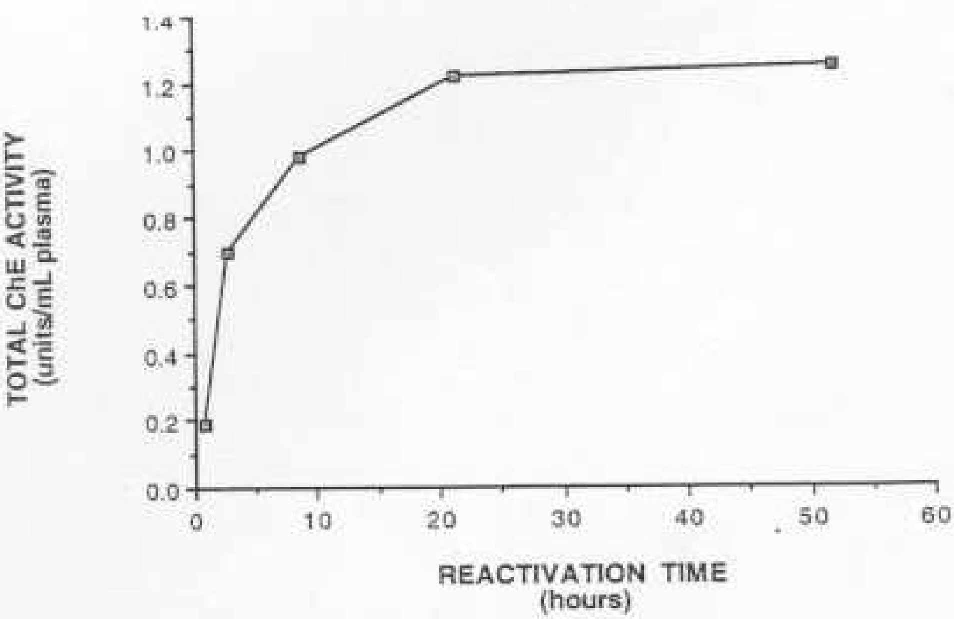

Fig. 2. The laughing gull plasma cholinesterase activity was reactivated over a period of 51 hours. ChE activity had been depressed 87 percent.

CHAPTER 4

ASSESSMENT OF A CARBAMATE APPLICATION TO A COASTAL GOLF COURSE ECOSYSTEM

ASSESSMENT OF A CARBAMATE APPLICATION TO A COASTAL GOLF COURSE ECOSYSTEM

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Abstract - The Ocean Course on Kiawah Island, South Carolina, was constructed with a unique drainage system allowing recycling of irrigation water. A tile drainage system installed under the course collects and channels water from greens and fairways into a series of man-made lagoons from which the water can be reused. This study is a component of an interdisciplinary project undertaken at the Ocean Course to assess the impact of pesticides on the surrounding golf course ecosystem. Following an application of bendiocarb, various endpoints were assessed including fish cholinesterase activities and residues present in turf, runoff, lagoon water, and aquatic plants. The limited amount of runoff contained approximately 1350 Fg/l active ingredient and resulted in little to no exposure in the lagoons. Approximately 16% of the active ingredient remained on the granules following the watering-in process. Bendiocarb levels in turf returned to below detection by four days post-application. A background level of bendiocarb was found in the aquatic plants sampled. Levels in the aquatic plants and turf increased following application of bendiocarb. Brain cholinesterase levels of fish in the lagoon were variable and exhibited no clear signs of carbamate exposure.

Keywords - Bendiocarb, *Ruppia maritima*, *Cyprinodon variegatus*, *Gambusia affinis*, turf, golf course

INTRODUCTION

Bendiocarb is a carbamate insecticide that was commonly applied to the Ocean Course, Kiawah Island, SC, in 1993. Because of its restricted use status, it is not commonly used on golf courses in South Carolina [1]. In order to simulate a "worst-case" scenario, bendiocarb 2.5 G was applied to a green on the Ocean Course and was then irrigated sufficiently to cause runoff into a surface drain which discharges into a nearby brackish lagoon. In this way, the maximum amount of chemical would be washed into the lagoon and the exposure and effects could be monitored.

The Ocean Course is one of four golf courses on Kiawah Island, SC, located just south of Charleston, SC. The course is bound by the Atlantic Ocean and tidal saltmarshes. Within the course proper, a brackish lagoon system receives runoff and drain water from the course=s sub-surface tile system. The seventh green is surrounded by sand dunes vegetated with sea oats and live oaks. The green funnels down to a drain linked to the nearby brackish canal. At the point of outflow, the drainage pipe was below water level and had to be cleared of sand prior to application. The outlet area was surrounded by cattails and widgeon grass (*Ruppia maritima*) in the canal. The average width and depth of the canal was 5 meters and 1 meter, respectively.

Turfgrass was monitored for bendiocarb residues, as certain waterfowl forage on turfgrasses and are susceptible to this route of exposure [2]. Aquatic and terrestrial plants were monitored for pesticide residues to determine if bendiocarb was concentrated in their tissues. The predominant aquatic macrophytes in the lagoons on the Kiawah Island Ocean Course are Cattails (*Typha sp.*) and widgeon grass. *R. maritima* is a rooted submerged plant and Cattails are rooted emergent plants. We utilized *R. maritima* for aquatic plant work in this study, as it is a known waterfowl food [3] and could be involved with food chain effects of pesticides.

Irrigation runoff and lagoon samples were collected to determine how much pesticide was transported and available in lagoon water. Sheepshead minnows (*Cyprinodon variegatus*), the most euryhaline fish species known, and mosquitofish (*Gambusia affinis*), also euryhaline but a live bearer, are common fish species found on the Ocean Course. Though euryhaline, mosquitofish populations choose bodies of water with low salinity. Both species are hardy and easily reared in a laboratory setting. There is a vast data base in the literature as to the effects of various xenobiotics and environmental factors on both fish species. It is interesting to note that mosquitofish populations in areas of chronic exposure to pesticides have shown the ability to develop resistance [4,5]. The acute toxicity (96h LC50) of bendiocarb for freshwater fish ranges from 0.4 to 1.5 Fg/l, but data is lacking for estuarine or marine organisms [6]. Brain cholinesterase (ChE) depression is well established as an indicator of carbamate or organophosphate insecticide exposure. Levels of brain cholinesterase in sheepshead minnows and mosquitofish were monitored for one week post-application so as to assess possible carbamate exposure to residing fish populations.

METHODS

Chemical application

Bendiocarb was applied to number 7 green on August 24, 1993 in a granular form at a target rate of 1.9 lb/1000 ft^2 active ingredient (AI). The chemical was applied using a broadcast spreader, avoiding overlap by marking the edge of the spread zone with flags. The green was then irrigated for 120 minutes at approximately 500 gallons/acre/minute. In order to determine the amount of bendiocarb applied and the amount remaining on the granules after irrigating, Pyrex and wire mesh containers were placed on the green to collect application samples pre- and post-irrigation. Turf samples were collected with a 1 cm cork borer at 0, 1, 2, 3, 4, and 7 days post application. Runoff samples were collected every 5 minutes once runoff began. Water and fish samples were collected in concert at 0, 1, 2, 4, 8, 16, 24, 48, 72, 96, and 168 hours. *R. maritima* samples were collected at 0, 1, 2, 4, 12, and 24 hours. *R. maritima* was also sampled in lagoons following insecticide applications throughout July 1993 (see Bailey et al., this report).

Environmental chemistry

Bendiocarb (Crescent Chemical Co., Hauppauge, NY), 99% purity, was used as an analytical reference standard and bendiocarb 2´ G (NOR-AM Chemical Co. Wilmington, DE; 2.5% active ingredient) was applied in the field. For turf analysis, 2-5 g of turf was mixed with 35 mL 75:25 pentane/ether and 2 g anhydrous sodium sulfate (oven dried at 130EC for 24 hrs) and placed on an orbital shaker, set at 250 rpm, for 30 minutes. After shaking, the pentane/ether was decanted through Whatman 41 filter paper containing 30 g of anhydrous sodium sulfate pre-wetted with pentane/ether. The sodium sulfate filter was rinsed three times with approximately 5 mL pentane/ether mixture. The sample was then rotary evaporated to dryness using a Büchi RE 111 Cold Finger Rotavapor with a water bath set at 40EC. Samples were brought to final volume in methanol.

Water samples were extracted using 3M C_{18} Bakerbond J Empore J disks (3M Inc., St. Paul, MN) on a six place solid phase extraction manifold (Millipore Corp, Bedford, MA). The extraction procedure involved pre-conditioning the Empore J disk with 5 ml of ethyl acetate and then aspirating the disk for 5 min. Next, 10 ml of methanol was added to the disk followed by 10 ml distilled water and then the water sample. After the water sample had passed through the disk, vacuum was maintained for 5 min. The sample was eluted by pulling 5 ml of a 10 ml aliquot of ethyl acetate through the disk. The vacuum was turned off, allowing the disk to soak for 1 min after which the remaining ethyl acetate was eluted. The elution step was repeated twice. Samples were concentrated under a constant flow of nitrogen using a Meyer N-Evap with glass bead bath set at 50EC (Organomation Assoc., Berlin, MA). Samples were brought to final volume in methanol.

For pesticide residue analysis, aquatic plants were finely chopped with scalpels and mixed with 35 ml 25:75 acetone/hexane and 2 g anhydrous sodium sulfate (oven dried at 130EC for 24 hrs) and placed on an orbital shaker, set at 250 rpm, for 30 minutes. After shaking, the acetone/hexane was decanted through Whatman 41 filter paper containing 30 g of anhydrous sodium sulfate pre-wetted with acetone/hexane. The sodium sulfate filter was rinsed three times with approximately 5 ml acetone/hexane mixture. The sample was then rotary evaporated to dryness using a Büchi RE 111 Cold Finger Rotavapor with a water bath set at 40EC. Samples were brought to final volume in methanol.

Bendiocarb was quantitated by a Hewlett Packard 1090 HPLC equipped with a post column reaction module (PCK 5000, Pickering Laboratories), and a Hewlett Packard 1046 A fluorescence detector. A 15.0 cm C18 column with 5 Fm packing was used (Pickering Laboratories) with a flow rate of 1.0 ml/min methanol/water mobile phase. A solvent gradient was used to separate the analytes of interest. The initial solvent mixture was 10:90 MeOH:water which was maintained for 0.5 min., the solvent mixture was adjusted to 50:50 over 12 min and then adjusted to 100% methanol over 0.5 min. After 3 min the solvent ratio was returned to 10:90 over 1.5 min. The bendiocarb was derivatized with *o*-phthalaldehyde and measured by fluorescence detection using 340 nm excitation and 455 nm emission wavelengths.

Brain cholinesterase assays

Levels of brain ChE in sheepshead minnows and mosquitofish were monitored for one week post-application so as to assess possible carbamate exposure to residing fish populations. Indigenous adult sheepshead minnows (*Cyprinodon variegatus*, n=24) and mosquitofish (*Gambusia affinis*, n=18) were seine netted from the study site 2 hours before the application and placed in cages. Laboratory-reared sheepshead minnows (n=32) were transported to the study site and placed in cages prior to application. The cages were 24@x18@x10@ in dimension and fitted with floats, allowing a one inch head space. The cages were placed six feet from the outflow. Fish of each species and strain were collected coinciding with water sampling, placed on dry ice and stored at -80EC until preparation for analysis. Each brain was removed from frozen carcasses, diluted 100-fold and homogenized in a 0.1 M Trizma (7.4 pH) buffer solution. The brains were homogenized with a Wheaton grinding chamber and Teflon7 pestle (Curtin Matheson Scientific, Inc., Atlanta, GA). Samples were kept on wet ice during preparation and were then assayed immediately to determine initial total ChE activity using the method of Ellman et al. [7] as modified by Hooper et al. [8] for use on a UVMax kinetic microplate spectro-photometer (Molecular Devices Corp., Palo Alto, CA). The change in absorbance was converted to micro moles substrate hydrolyzed per minute per gram brain tissue. Assay concentration of the colorimetric agent 5,5=-dithiobis-2-nitrobenzoic acid (DTNB) was 3.23 X 10^{-3} M. The substrate for enzyme action was acetylthiocholine iodide (AThCh). Substrate concentration was 1 X 10^{-3} M for 32 laboratory sheepshead minnows and all 18 mosquitofish analyses. AThCh concentration for the 24 indigenous sheepshead minnows was 4 X 10^{-4} M. Levels of CA-inhibited ChE were determined using a dilution technique for promoting the reversibility of the carbamate-enzyme bond [9,10]. Samples were divided into two equal aliquots, one of which was incubated at 1EC on wet ice and the other at 37EC. All samples were analyzed after 4 hours incubation. Some samples were also analyzed at 1, 2 and 8 hours. All post-incubation ChE activities were compared to their respective initial activities. Samples found to have a significant increase in activity of at least 5% were considered exposed to a CA. All enzyme activities were measured in triplicate. A one-tailed Student's *t*-test was used to determine if ChE activities at 37EC were significantly (p <0.05) increased over activities at 1EC. All reagents were purchased from Sigma Chemical Company, St. Louis, MO.

RESULTS AND DISCUSSION

Application

Knowing the surface area of the containers, the amount of bendiocarb applied was calculated to be 1.90 lb/acre with 0.3 lb/acre remaining after irrigation. This shows that 16% of the active ingredient remains on the granule after irrigation. The chemical remaining on the granule is available for potential exposure to wildlife foraging on the green.

Turf

To determine possible nontarget pesticide exposure to wildlife, turf patches from the course were analyzed for bendiocarb residues. After the August 24, 1993 monitored application of bendiocarb to the seventh green, the chemical was found in the turf at 24 hours and was seen to decline to below detection limits over 4 days (Figure 1).

Runoff

Runoff from the green occurred for a total of 25 minutes. Samples were collected continually for 5 minute intervals. Since runoff volume was limited, sample collection resulted in approximately 1/4 of the total runoff flow being collected for analysis. As a result of the large portion of runoff collected as sample, we calculated total bendiocarb delivered to the system as 3 times the amount collected (1/4 collected, 3/4 delivered to system). The result being approximately 1350 Fg/l active ingredient delivered to the system and total volume 1.2 liters.

Lagoon Water

Water samples from the lagoon showed no detectable levels of bendiocarb. This is attributed to the low amount of runoff and the large dilution of runoff between the drain and lagoon.

Aquatic plants

We collected aquatic plants for residue analysis from Ocean Course lagoons in conjunction with insecticide applications, to determine the mobility and bioavailability of these chemicals in the tile drain and lagoon system.

Chemical analysis of *R. maritima* samples taken in conjunction with insecticide applications (bendiocarb, chlorpyrifos) to the Ocean Course showed only detectable levels of Bendiocarb (see Bailey et al., this report). Bendiocarb residues are highest immediately after application and decrease over time. Since *R. maritima* is an aquatic plant, these data indicate bendiocarb does reach the lagoons on the course, leading to possible foodchain uptake. However, since the bendiocarb LD50 value for mallards is 3.1mg/kg [11], an average mallard weighing approximately 1 kg [12] would have to eat 10,000g of plant material to reach an acutely toxic dose. This makes ecological relevance of exposure to *R. maritima* undetermined. Other chemicals applied are either not accumulated by *R. maritima* or they are not reaching the lagoons in detectable amounts. After the August 24 application, bendiocarb was seen in *R. maritima* (113 ng/g) from the outlet lagoon at the 2 hour timepoint, but none of the others.

Fish

The brain ChE activity of individual sheepshead minnows collected post-application varied relative to time of collection. When the assay concentration of AThCh was 1×10^{-3} M, individual ChE activities ranged from 1.340 to 18.388 units/g brain tissue of laboratory fish. Group means decreased from 14.422 units/g at 1 hour post-application to a low of 2.818 units/g at 96 hours. By 168 hours, mean ChE activity had recovered to 9.641 units/g. Similar results were obtained with an AThCh concentration of 4×10^{-4} M. Individual activities of wild fish ranged from 1.859 to 18.267 units/g. Group means decreased from 14.101 at 1 hour to a low of 2.645 units/g at 96 hours. Wild mosquitofish brain ChE ranged from 2.767 to 13.283 units/g brain tissue. Mean activity decreased from 13.283 units/g at one hour to 2.767 units/g at 8 hours. Samples were lost for the 96 hour time point. Figure 2 shows the mean brain ChE activity of the fish over the sampling period.

The reactivation assay resulted in 9 (16%) of 56 sheepshead minnow samples which showed at least 5% reactivation after 4 hours incubation. Of these, six were samples at 96 hours post application. Only one laboratory fish exhibited reactivation (6.7%). The mean reactivation of the five wild fish at 96 hours post application was 16.8%. None of the mosquito-fish sampled showed reactivation greater than 5%.

CONCLUSIONS

There is potential for exposure to wildlife from pesticide remaining on granules after the chemical is "watered in". Bendiocarb remains in the turf for a relatively short period of time, leading to a small window for non-target pesticide exposure. Although there was runoff entering the drain, there was no measurable impact on the lagoon organisms. Brain cholinesterase levels of fish in the lagoon were variable and exhibited no clear signs of carbamate exposure.

Since the chemical did not reach the lagoon in detectable amounts, it appears that there is little opportunity for possible toxicity to occur in the aquatic systems. And, since this was a worst case scenario, it is quite possible that under normal conditions with no surface runoff even less chemical reaches the lagoon system. In fact, soil column studies (Cowles et al., this report) designed to mimic the conditions on Ocean Course greens showed no movement of bendiocarb past 10 cm into the column over a 5 day period.

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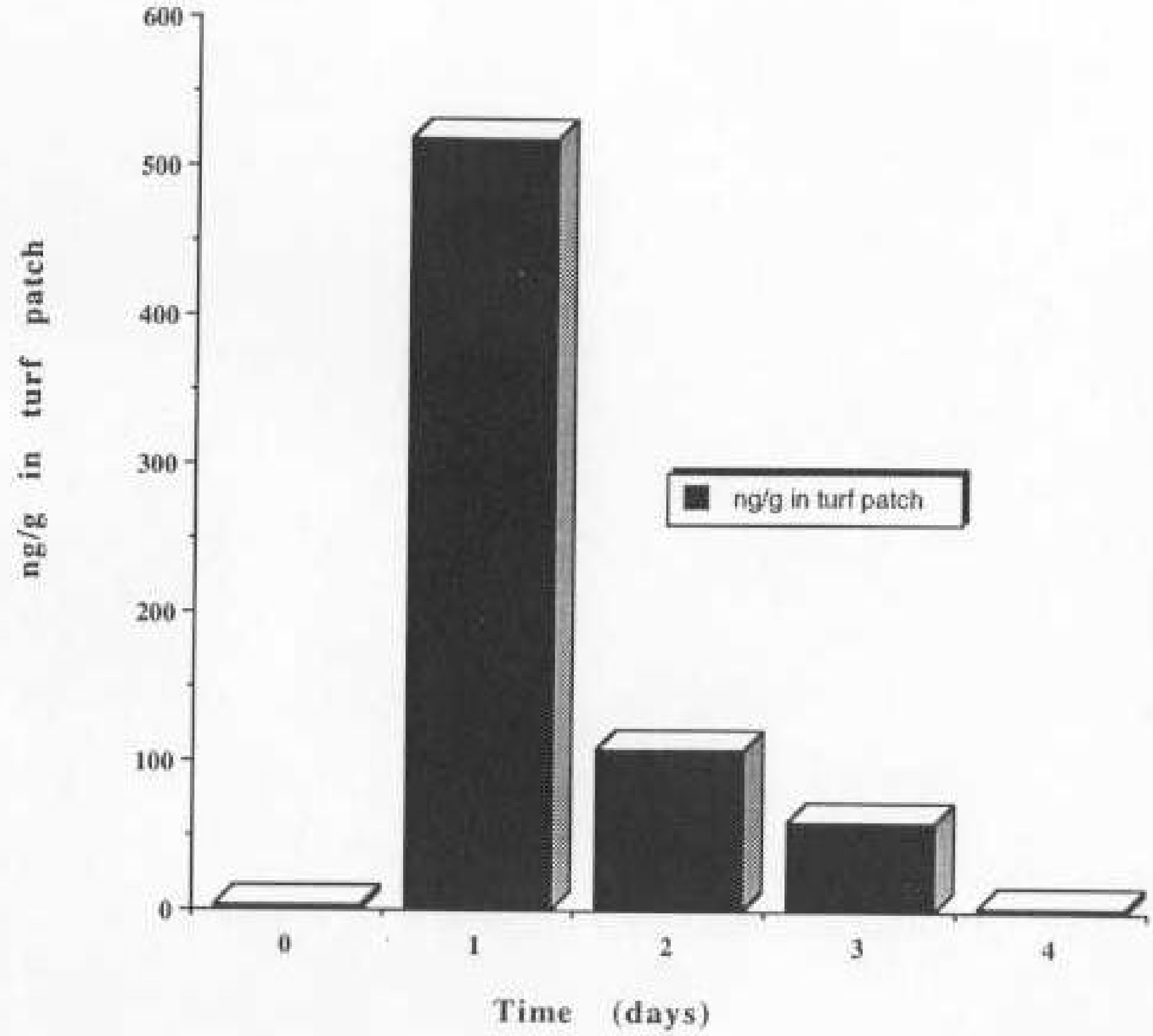

Figure 1. Bendiocarb in turf after August 24, 1993 application to green #7.

Figure 1. Bendiocarb in turf after August 24, 1993 application

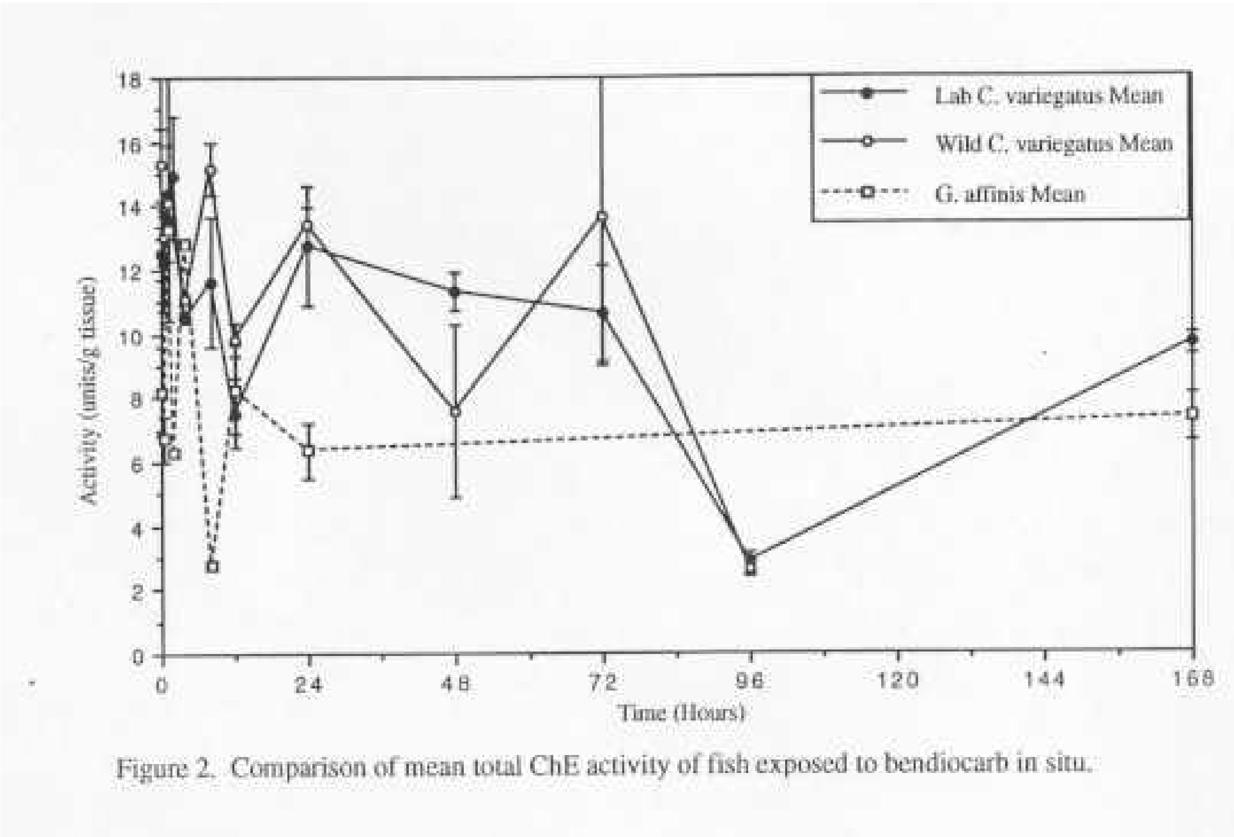

Figure 2. Comparison of mean total ChE activity of fish exposed to bendiocarb *in situ*

CHAPTER 5

DETERMINATION OF PESTICIDE MOVEMENT OF CHLORPYRIFOS AND BENDIOCARB THROUGH TWO SOIL MATRICES

MOVEMENT OF CHLORPYRIFOS AND BENDIOCARB THROUGH TWO SOIL MATRICES

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Abstract - To determine the environmental fate of pesticides applied to golf courses, flow dynamics and system drainage must be understood. Soil columns have traditionally been used to model chemical movement in specific soil types over time. It is important to know the residence time of analytes in drainage systems to assess the ultimate fate of chemicals. The United States Golf Association (USGA) specifies soil mix profiles for golf greens as well as the design of subsurface drainage systems. It is necessary to characterize pesticide residence time in these systems to determine potential exposure to wildlife in areas receiving effluent from them. We constructed artificial soil columns with soil mixtures that simulated a golf course putting green on the Ocean Course, Kiawah Island, SC. Pesticide retention in the Ocean Course root zone mixture was compared to a mixture made offsite which conforms to the revised USGA (1993) root zone criteria. We examined chlorpyrifos and bendiocarb mobility through the root zone mixtures using the constructed soil columns. Soil columns were constructed with and without a turf patch to evaluate the effects of thatch on pesticide mobility. Batch adsorption studies were conducted to characterize partitioning of these compounds in the different soil types used. We found that chlorpyrifos did not move significantly through either soil mix, with or without turf. Bendiocarb migration was significantly reduced by turf/thatch in both soil types. Bendiocarb degradation appears to be enhanced in the Kiawah root zone mix, making calculation of K values suspect.

Keywords - Soil column Bendiocarb Chlorpyrifos Turf

INTRODUCTION

The Ocean Course on Kiawah Island, South Carolina, was constructed with a unique drainage system allowing recycling of irrigation water. A tile drainage system installed under the course collects and channels water from greens and fairways into a series of man-made lagoons from which the water can be reused. This study is a component of an interdisciplinary project undertaken to assess the impact on the surrounding environment of golf course management techniques implemented at the Ocean Course. Various endpoints were assessed including avian and fish toxicity and nutrient cycling. To assess potential pesticide mobility in the Ocean Course drainage system, we compared the Ocean Course root zone mix to one prepared offsite according to USGA criteria [1]. The Ocean Course root zone mix was prepared onsite using native sand.

Chlorpyrifos and bendiocarb were evaluated because of their extensive use on the Ocean

Course. Batch sorption studies were conducted to determine distribution coefficients for chlorpyrifos and bendiocarb in the two root zone mixtures.

Soil column experiments were conducted to evaluate pesticide mobility through a green structure similar to that of the Ocean Course using both root zone mixes. The composition of the Ocean Course greens was determined by taking soil cores from several greens. In addition, a second set of experiments was conducted evaluating leachability of the two root zone mixes.

EXPERIMENTAL SECTION

Materials

Chlorpyrifos (U.S. Environmental Protection Agency; 99.3% purity) and bendiocarb (Crescent Chemical Co., Hauppauge, NY; 99% purity) where used as analytical reference standards and in the batch adsorption studies. Formulations of chlorpyrifos (Chemisco, St. Louis, MO; 6.62% active ingredient) and bendiocarb 2.5 G (NOR-AM Chemical Co. Wilmington, DE; 2.5% active ingredient) were used for the soil column experiments.

Root zone mixtures were obtained from The Ocean Course, Kiawah Island, SC and Murray Sand Co. Summerville, SC. The physical characteristics of the Murray and Kiawah root zone mixes were determined by Turf Diagnostics and Design, Olathe, Kansas. The root zone mixes were evaluated using the 1993 United States Golf Association (USGA) testing protocol [2] and the results compared to the 1993 USGA recommendations for putting green construction [3].

Extraction and Analysis

Soil samples were extracted according to TIWET SOP-401-27-02 (Appendix 1). Briefly, 2-5 g of soil was mixed with 35 ml 75:25 pentane/ether and 2 g anhydrous sodium sulfate (oven dried at 130EC for 24 hrs) and placed on an orbital shaker, set at 250 rpm, for 30 minutes. After shaking, the pentane/ether was decanted through Whatman 41 filter paper containing 30 g of anhydrous sodium sulfate pre-wetted with pentane/ether. The sodium sulfate filter was rinsed three times with approximately 5 ml pentane/ether. The sample was then rotary evaporated to dryness using a Büchi RE 111 Cold Finger Rotavapor with a water bath set at 40EC. Samples were brought to final volume in hexane or methanol for chlorpyrifos and bendiocarb respectively.

Water samples were extracted using 3M C18 Bakerbond J Empore J disks (3M Inc., St. Paul, MN) on a six place solid phase extraction manifold (Millipore Corp, Bedford, MA). The extraction procedure involved pre-conditioning the Empore J disk with 5 ml of ethyl acetate and then aspirating the disk for 5 min. Next, 10 ml of methanol was added to the disk followed by 10 ml distilled water and then the water sample. After the water sample had passed through the disk, vacuum was maintained for 5 min. The sample was eluted by pulling 5 ml of a 10 ml aliquot of ethyl acetate through the disk. The vacuum was turned off, allowing the disk to soak for 1 min after which the remaining ethyl acetate was eluted. The elution step was repeated twice. Samples were concentrated under a constant flow of nitrogen using a Meyer N-Evap with glass bead bath set at 50EC (Organomation Assoc., Berlin, MA). Samples were brought to final volume with hexane or methanol chlorpyrifos and bendiocarb respectively.

Chlorpyrifos concentrations were determined on a Hewlett Packard 5890 Series II gas chromatograph with a DB-1 column (30 m x 0.53 mm ID, 1.5 ìm coating, J & W Scientific. Folsom, CA), flame photometric detector, and a Hewlett Packard 7673 auto sampler. The injection port and detector temperatures were set at 250EC and 300EC, respectively, and the oven temperature was 140EC for 0.5 min raised to 200EC at 25EC/min, then raised at 10EC/min to 250EC which was held for 5 min. The carrier gas flow of UHP Helium (Air Products, Allentown, PA) was set at 10.9 mL/min at 140EC.

Bendiocarb was quanitated by a Hewlett Packard 1090 HPLC equipped with a post column reaction module (PCK 5000, Pickering Laboratories), and a Hewlett Packard 1046 A fluorescence detector. A 15.0 cm C18 column with 5 ìm packing was used (Pickering Laboratories) with a flow rate of 1.0 ml/min methanol/water mobile phase. A solvent gradient was used to separate the analytes of interest. The initial solvent mixture was 10:90 MeOH:water which was maintained for 0.5 min., the solvent mixture was adjusted to 50:50 over 12 min and then adjusted to 100% methanol over 0.5 min. After 3 min the solvent ratio was returned to 10:90 over 1.5 min. The bendiocarb was derivatized with *o*-phthalaldehyde and measured by fluorescence detection using 340 nm excitation and 455 nm emission wavelengths.

Batch Sorption Studies

Soils used for the sorption studies were dried at 100EC until a constant weight was obtained. Teflon centrifuge tubes were fortified with chlorpyrifos or bendiocarb and the carrier solvent evaporated under a stream of nitrogen. 10.0 ml of distilled water was added to the centrifuge tube followed by 2 g of soil. Solid to solution ratios were maintained at 1:5 for all sorption experiments [4]. The centrifuge tubes were placed on an orbital shaker for 24 hrs at 250 rpm. Unfortified and fortified control samples of water and soil were also prepared. After shaking, the samples were centrifuged for 5 min using an IEC bench top centrifuge to separate the aqueous and solid phases. The two phases were then separated by decanting the aqueous phase, which was extracted as previously described. The aqueous phase was analyzed, and the remaining chemical was assumed to be in the soil fraction. Distribution coefficients (K_d) were calculated as the ratio of analyte concentration in the solid (ng/g) and liquid (ng/mL) phases [5].

Soil Columns Studies

Two soil column experiments were conducted. Figure 1 illustrates soil column construction. Distilled water was percolated through the columns as they were filled to prevent air pockets from forming [6].

The first experiment evaluated the effects of turf on pesticide migration through the Ocean Course and Murray root zone mixtures. To simulate a worst case scenario, the amount of water applied to the soil columns was twice the volume applied during normal irrigation at the Ocean Course.

Once the soil column was filled, the water level was lowered to just below the root zone mix to simulate the saturation zone conditions found on the course. Chlorpyrifos and bendiocarb were then applied simultaneously at label rates and washed into the turf patch with 120 ml of distilled water. After the water percolated through the column to the existing water level, 120 ml of water was drained and collected from the column. In the same manner, 120 ml of distilled water was added and collected for 5 consecutive days. The soil column was then dismantled, and a soil sample was collected every 10 cm in depth for residue analysis. Duplicate columns of each soil type were run.

The second soil column experiment evaluated pesticide migration through the two root zone mixes. The turf patch was omitted and the water level was lowered approximately 2 cm below the surface of the soil. Chlorpyrifos and bendiocarb were then applied at the label rate. The water level was raised to provide a constant hydraulic head of 10 cm. A constant flow of approximately 0.6 ml/min was maintained in each column. Duplicate columns of each soil type were run from which soil samples were collected at 5 cm intervals for residue analysis at the end of five days.

RESULTS AND DISCUSSION

Soil Physical Characteristics

The particle size analysis of the Ocean Course root zone mix determined that the sample contained 4.4% more material on the 270 mesh sieve than is recommended by the USGA criteria. The other sand fractions for the Ocean Course were within the USGA recommended ranges. The physical evaluation of the Ocean Course mix met the USGA performance criteria. The organic matter content (dry weight basis) was lower than USGA minimum recommendation of 1.0%. The bulk density of the mix was high indicating the potential for a hard surface. The Ocean Course mix had a slight acid reaction indicating a calcareous sand [7]. Calcareous sands may exhibit accelerated weathering leading to the possibility of the green structure deteriorating [8].

The Murray Sand Co. mix fit the USGA criteria for particle size. The physical performance of the Murray mix met the USGA recommendations although the infiltration rate was in the accelerated range. Although the infiltration rate of the Murray mix was high, the capillary porosity indicated that the mix held the minimum amount of water necessary [7].

A complete listing of soil characteristics for the Ocean Course and Murray Sand Co. root zone mixes is in Appendix 2.

Batch Sorption Studies

Theory

Freundlich sorption isotherms were constructed (Figures 2 and 3) from which K and K_{oc} values were calculated (Table 1). Representative literature values of K_{oc}, water solubility and environmental half-life in soil for bendiocarb and chlorpyrifos are listed in Table 2. The Freundlich isotherm is based on the linear equation

$$\log \frac{x}{m} = \log K + \frac{1}{n} \log C \quad 1$$

were x/m equals ng/g sorbed at equilibrium, K is the partition coefficient, 1/n is the degree of linearity and C is ng/mL at equilibrium. K values were calculated following

$$K = \frac{x/m}{C} \quad 2$$

assuming 1/n to be equal to one and at a value of C within our experimental range. K_{oc} values were calculated as

$$K_{oc} = \frac{K}{f_{oc}} \quad 3$$

where f_{oc} is the fraction of organic carbon for the soil type.

If the distribution of a compound is driven strictly by organic carbon content of the soil, then K_{oc} should be consistent for a compound across soil types. Root zone mixtures have a very high sand content and low organic carbon in comparison to conventional soils. As a result, the assumptions for comparing pesticide mobility through root zone mixes based on K_{oc} may be in error. Other factors affecting sorption in root zone mixes are clay content, cation exchange capacity, particle size distribution, solids to solution ratio, and loss of chemical during the test. In order for the assumptions of the Freundlich isotherm to be valid 1/n must be close to one. Generally, linearity values of 0.7 to 1.1 are found although values of 0.3 to 1.7 are reported in the literature [9]. The deviation from linearity (1/n) of the isotherm is also important to the calculation of K_{oc} based on K.

Chlorpyrifos

The different K_{oc} values for chlorpyrifos in the two root zone mixes indicate factors other than soil organic carbon content must be influencing adsorption. Also, the linearity term (1/n) was greater than one for chlorpyrifos in both root zone mixes causing the K_{oc} values to be dependent on the range of concentrations tested.

Bendiocarb

While K_{oc} values for bendiocarb are consistent between soils and with literature values, the values are suspect because we found no residues of the compound in the Ocean Course soil fraction. Tests with fortified soils indicated recovery of bendiocarb was possible. We therefore believe that the lack of detection of bendiocarb is due to degradation rather than irreversible binding or analytical difficulties. It is likely that the Ocean Course mix, which has a high portion of fine particles, binds the chemical and causes a rapid binding-induced hydrolysis [10]. If this is true, then the assumption of equilibrium in the batch adsorption experiments for bendiocarb on the Ocean Course mix is in error. The practice of using only water residue data and assuming that the rest of the chemical is in the soil fraction to calculate K also appears to be invalid in this case.

Soil Column Studies

Chlorpyrifos

In experiment one, chlorpyrifos was found only in the first 10 cm of the soil column, which consisted of the turf patch. Low mobility of chlorpyrifos through the turf on the soil columns indicates that this compound should pose minimal risk to the surrounding ecosystem.

In experiment two, chlorpyrifos was only detected in the upper 5 cm of the soil column for both root zone mixes. Low mobility of chlorpyrifos in turf and root zone mixes was predicted by the K_{oc} values calculated from the batch sorption studies. A large K indicates a greater affinity for the soil fraction than the mobile water phase. Of interest is the low mobility in the Ocean Course root zone mix despite the low OC (0.5%) content.

Fermanich and Daniel found similar results while studying pesticide mobility in sandy soil columns in which less than 0.2% of chlorpyrifos applied leached and greater than 78% of chlorpyrifos recovered from soil was found in the upper 5 cm [11].

Bendiocarb

In experiment one, bendiocarb was found only in the first 10 cm of the soil column, which consisted of the turf patch. As with chlorpyrifos, bendiocarb under these conditions should pose minimal risk for movement to the surrounding ecosystem. Results of the bendiocarb soil column experiments indicate the inherent danger of predicting the environmental fate of a compound based solely on partition coefficients. Based on calculated and published K_{oc} values, it is expected that bendiocarb would readily move through the turf and soil column.

In experiment two, bendiocarb was detected in water samples collected from the Murray root zone mix but was below detection limits in the soil samples. Bendiocarb was not detected in any of the soil or water fractions of the Ocean Course root zone mix.

The absence of bendiocarb in the Ocean Course mix is consistent with analytical results obtained from sorption studies. We believe that increased degradation of bendiocarb in the Ocean course mix is due to two processes: 1) bendiocarb maybe undergoing increased hydrolysis as a result of binding to the increased surface area of the fine particle fractions; 2) bendiocarb degradation may result from microbial populations present in the Ocean Course root zone mix utilizing the bendiocarb as a carbon source. It has been shown that microbial populations in soils previously exposed to carbamate pesticides can rapidly degrade carbamate pesticides [12, 13]. Since the history of the Ocean Course root zone mix is unknown, it is possible the root zone mix has been previously treated with a carbamate pesticide.

CONCLUSIONS

Chlorpyrifos does not migrate significantly through the turf or root zone mixes tested and should pose little risk to the surrounding environment due to its low mobility. The low mobility of chlorpyrifos found in our study is consistent with previous work [11,14,15]. Runoff of chlorpyrifos can be further minimized by applying its granular formulation which has been shown to deliver more active ingredient to the soil than the liquid formulation [15]. Bendiocarb did not migrate through the turf but did migrate through both root zone mixes tested. Bendiocarb should pose minimal risk to the surrounding ecosystems as long as it is applied only to turf. Care should be taken to prevent over-spray into sand traps and onto surrounding dunes.

ACKNOWLEDGEMENTS

This project was sponsored by the United States Golf Association (USGA), Monsanto, and Pro Golfers Association of America (PGA). In addition the authors would like to thank the Murray Sand Co. of Summerville, SC for donating the root zone mix meeting USGA criteria.

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Table 1. Batch sorption results.

	Ocean Course mix		Murray mix	
	K	K_{oc}	K	K_{oc}
Chlorpyrifos	110	23,000	3100	190,000
Bendiocarb	3.0	560	7.0	430

Table 2. Literature parameters of chlorpyrifos and bendiocarb. ([y][16], [l][17])

	H_20 solubility[y] ppm	K_{oc}	t 2 soil[l]
Chlorpyrifos	2	6070	30 days
Bendiocarb	40	570	5 days

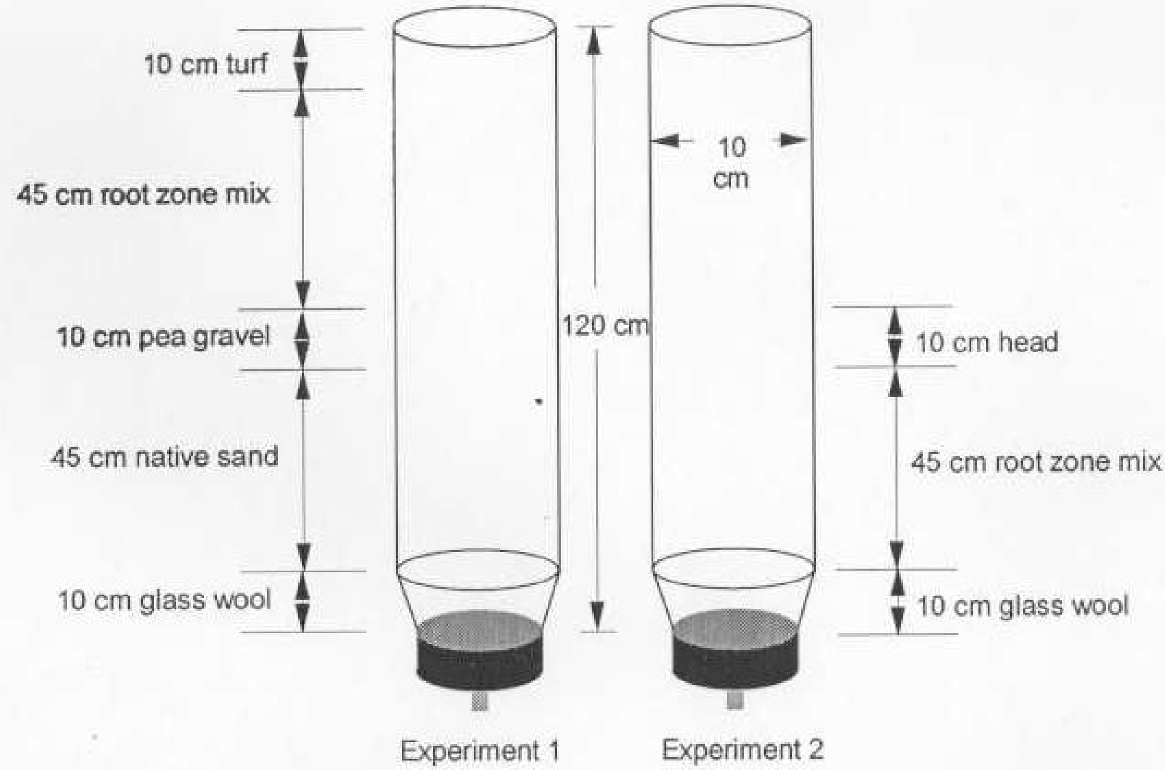

Figure 1. Soil column composition.

Figure 1. Soil column composition

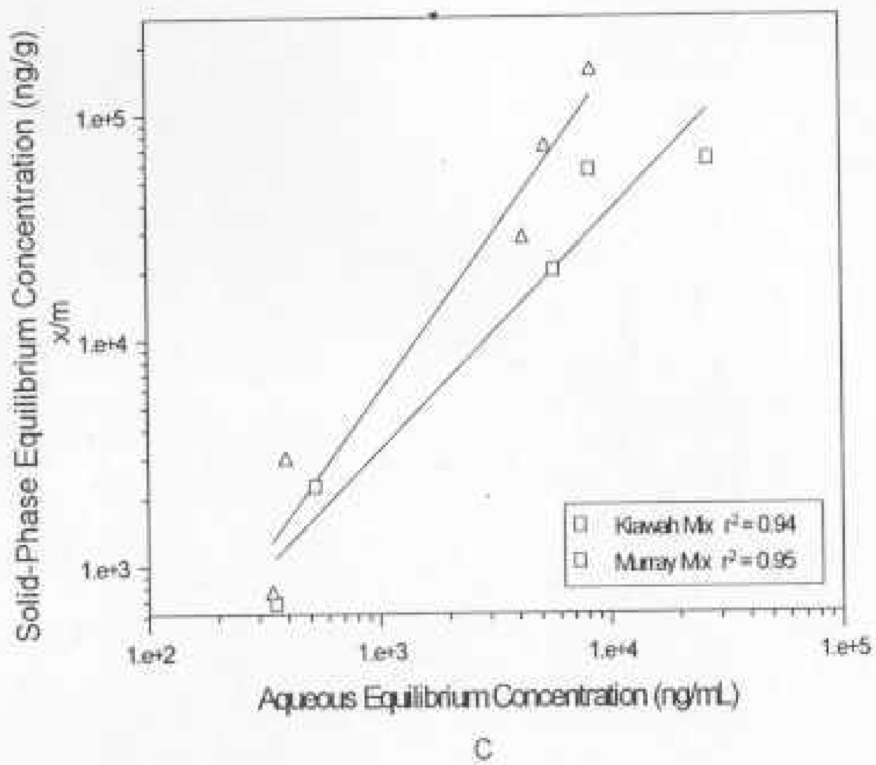

Figure 2. Bendiocarb sorption isotherm.

Figure 2. Bendiocarb sorption isotherm

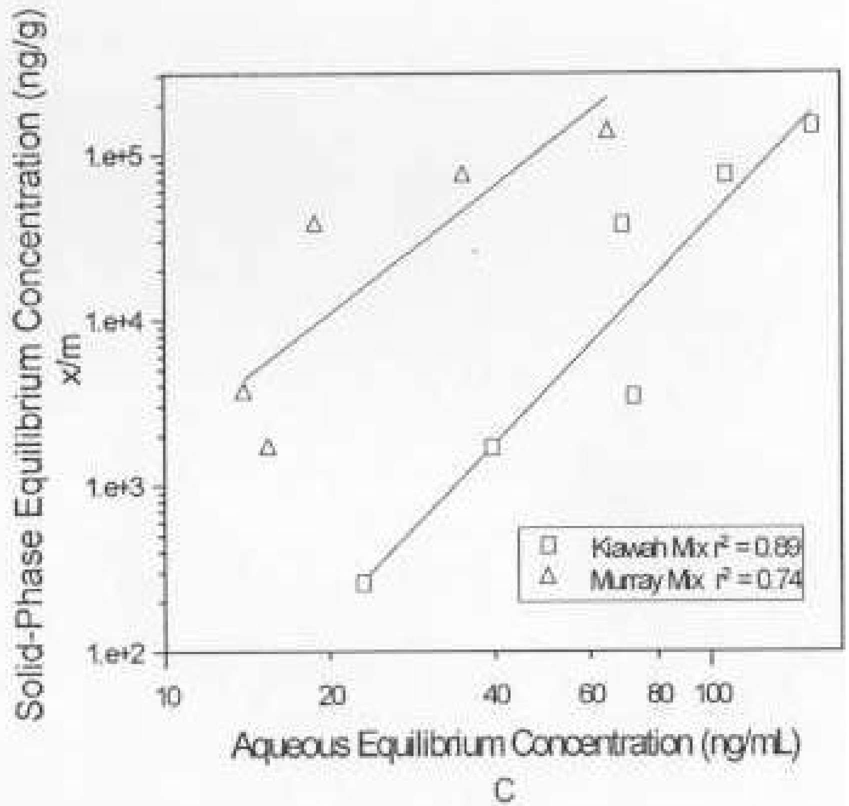

Figure 3. Chlorpyrifos sorption isotherm.

Figure 3. Chlorpyrifos sorption isotherm

CHAPTER 6

GOLF COURSE CHEMICAL MANAGEMENT PRACTICES AND THEIR EFFECTS ON SHEEPSHEAD MINNOWS (*Cyprinodon variegatus*) AND MOSQUITOFISH (*Gambusia affinis*), A COMPARISON OF *IN SITU* AND LABORATORY DATA

GOLF COURSE CHEMICAL MANAGEMENT PRACTICES AND THEIR EFFECTS ON SHEEPSHEAD MINNOWS (*Cyprinodon variegatus*) AND MOSQUITOFISH (*Gambusia affinis*), A COMPARISON OF *IN SITU* AND LABORATORY DATA

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Abstract - Present golf course management practices on the Ocean Course, Kiawah Island, SC, call for the use of various organophosphate and carbamate pesticides such as: acephate (Orthene**7**), chlorpyrifos (Dursban**7**), and bendiocarb (Turcam**7**). In addition, a significant volume of fertilizers are also applied to the course. Sheepshead minnows (*Cyprinodon variegatus*) and mosquitofish (*Gambusia affinis*) are two conspicuous species of fish found in the course's lagoon system. Laboratory acute toxicity tests were performed with chlorpyrifos and bendiocarb, using sheepshead minnows as an indicator species. An estimated 96h LC5O for chlorpyrifos and bendiocarb could not be determined with the results obtained. Brain cholinesterase activity was used to compare the response of fish dosed in the laboratory and that of those exposed to bendiocarb on the course via a "worst case" scenario artificially induced. Results indicate little risk to fish from the use of Dursban**7** or Turcam**7** on the Ocean Course, Kiawah Island, SC.

Keywords - *Cyprinodon variegatus* *Gambusia affinis* Chlorpyrifos Bendiocarb

INTRODUCTION

The proposed study of golf course chemical-use management is intended to address the effects of the current practices invoked on the Ocean Course (Kiawah Island, SC) on sheepshead minnows (*Cyprinodon variegatus*) and mosquitofish (*Gambusia affinis*). Present management practices call for the use of various organophosphate insecticides such as acephate (Orthene**7**) and chlorpyrifos (Dursban**7**). A particular carbamate, bendiocarb (Turcam**7**), and a significant volume of fertilizers are also applied to the course.

Sheepshead minnows, the most euryhaline fish species known, and mosquitofish, also euryhaline but a livebearer, are common fish species found on the Ocean Course. Though euryhaline, mosquitofish populations choose bodies of water with low salinity. Both species are hardy and easily reared in a laboratory setting. There is a vast data base in the literature as to the effects of various xenobiotics and environmental factors on both fish species. An interesting note on the selection of mosquitofish; populations in areas of chronic exposure to pesticides have shown the ability to develop resistance [1,2].

The mechanistic toxicity of carbamates and organophosphates (OP's) is well understood. Most notable are the studies of OP's inhibition of AChE. However, there are other effects described in the literature. Acephate has been shown to increase ventilation and buccal amplitude at levels slightly above the 4-8h LC5O [3]. OP's have been shown to cause skeletal deformities in fish [4]. Parathion-contaminated food was avoided by mullet [5]. It has been reported that organic pesticides cause pregnant *Gambusia* to abort embryos [6]. Abnormalities in the circulatory system were seen in medaka embryos exposed to parathion and malathion [7]. The runoff of OP's may have an indirect effect on the fish, in that invertebrates utilized as a food source may be severely impacted causing a nutritional deficiency. Table 1 exhibits the acute toxicity (96h LC5O) of some of the xenobiotics that are found on the Kiawah site to common North American freshwater fish [8].

The toxicity of chlorpyrifos, both lethal and sublethal, has been documented for several species of aquatic organisms [9-13]. Clark et al. [14] reports a 96h LC5O for the sheepshead minnow of 136 Fg/l. Mosquitofish appear to be less sensitive to chlorpyrifos, with a 96h LC5O of 340 Fg/l [15]. Bendiocarb, due to its restricted use status, is not commonly used and currently a small environmental toxicology database exists. The acute toxicity for freshwater organisms is less than that of chlorpyrifos and data is lacking for estuarine or marine organisms [16].

There are five environmental conditions that are important to consider when looking at pesticide toxicity. Increases in temperature cause an increase in metabolic rate and ventilation. This increased ventilation obviously increases the chance of uptake of water soluble toxicants. Reports are conflicting as to whether this causes increased toxicity or not because of the concurrent increase in metabolism of the xenobiotics. The hydrolysis of OP's is controlled by pH. Hardness has been shown to have little effect on OP toxicity [17]. A few studies have shown the role salinity plays in pesticide toxicity. Gambusia exhibited decreased accumulation of organic pesticides with increased salinity [18]. Very little has been done to characterize the part dissolved oxygen (DO) plays in this convoluted scheme [19].

Chlorpyrifos and bendiocarb were tested for acute toxicity (96h-LC5O) in the laboratory with both wild and laboratory raised *C. variegatus*. In an effort to establish a sublethal dose response, brain acetyl-cholinesterase levels were measured following the acute exposures. An *in situ* exposure to bendiocarb was assessed by monitoring brain AChE activities of feral and laboratory sheepshead minnows as well as wild mosquitofish.

MATERIALS AND METHODS

Site Assessment

Water chemistry data were collected bi-weekly in the recycling lagoon and the large lagoon on the back nine, near the #15 green (see Fouts et al., this report). Water samples were also taken and analyzed for nutrients (nitrates and phosphates) as well as pesticide residues. Seven species of fish were found on or around the course proper (Table 2). Fish were collected a with a 4'x30" (1/8" mesh) minnow seine. The tidally influenced creeks of the course maintained a mean salinity of 7 ppt. Portions of the creeks with higher salinity produced a different species composition. The higher salinity waters were inhabited mainly by sheepshead minnows (*Cyprinodon variegatus*), inland silverside (*Menidia beryllina*), striped mullet (*Mugil cephalus*), and sailfin molly (*Poecilia latipinna*). Mosquitofish (*Gambusia affinis*) was the dominant species in the lower saline environments. A few mummichogs

(*Fundulus heteroclitus*) were found on the front nine. Two dead carp (taxonomy unknown due to tissue decay) were collected on the front nine near #5 tee.

Acute Toxicity Tests

Juvenile sheepshead minnows were collected from the course and transported back to the laboratory. They were allowed to acclimate for two weeks prior to testing. Laboratory strain sheepshead minnows were obtained from the U.S. EPA laboratory at Gulf Breeze, FL. The chlorpyrifos tests were conducted with an EPA certified pure (97.8%) standard and the bendiocarb tests were conducted with standard grade (99.8% purity) bendiocarb (Ultra Scientific). Test vessels were made by partitioning 15 gallon aquaria with 1/4" plate-glass. Each vessel measured 12 cm x 30.5 cm x 30.5 cm and was filled with 4 liters reconstituted seawater [20] for testing. Five fish were placed in each vessel, with two vessels per treatment for a total of 10 fish per treatment. Five concentrations and a control were tested in order to bracket the LC5O value and result in partial kills. The nominal concentrations of chlorpyrifos tested were: 0, 50, 100, 200, and 1000 Fg/l. The nominal concentrations of bendiocarb tested were: 0, 50, 100, 500, 1000, and 5000 Fg/l.

In Situ Assays

Levels of brain ChE in sheepshead minnows and mosquitofish were monitored for one week post-application so as to assess possible carbamate exposure to residing fish populations. Indigenous adult sheepshead minnows (*Cyprinodon variegatus*, n=24) and mosquitofish (*Gambusia affinis*, n=18) were seine netted from the study site 2 hours before the application and placed in cages. Laboratory-reared sheepshead minnows (n=32) were transported to the study site and placed in cages prior to application. The cages were 24@x18@x10@ in dimension and fitted with floats, allowing a one inch head space. The cages were placed six feet from the outflow. Fish of each species and strain were collected coinciding with water sampling, placed on dry ice and stored at -80EC until preparation for analysis. Each brain was removed from frozen carcasses, diluted 100-fold and homogenized in a 0.1 M Trizma (7.4 pH) buffer solution. The brains were homogenized with a Wheaton grinding chamber and Teflon7 pestle (Curtin Matheson Scientific, Inc., Atlanta, GA). Samples were kept on wet ice during preparation and were then assayed immediately to determine initial total ChE activity using the method of Ellman et al. [21] as modified by Hooper and Wilson [22] for use on a UVMax kinetic microplate spectro-photometer (Molecular Devices Corp., Palo Alto, CA). The change in absorbance was converted to micro moles substrate hydrolyzed per minute per gram brain tissue. Assay concentration of the colorimetric agent 5,5=-dithiobis-2-nitrobenzoic acid (DTNB) was 3.23 X 10^{-3} M. The substrate for enzyme action was acetylthiocholine iodide (AThCh). Substrate concentration was 1 X 10^{-3} M for 32 laboratory sheepshead minnows and all 18 mosquitofish analyses. AThCh concentration for the 24 indigenous sheepshead minnows was 4 X 10^{-4} M. Levels of CA-inhibited ChE were determined using a dilution technique for promoting the reversibility of the carbamate-enzyme bond [23]. Samples were divided into two equal aliquots, one of which was incubated at 1EC on wet ice and the other at 37EC. All samples were analyzed after 4 hours incubation. Some samples were also analyzed at 1, 2 and 8 hours. All post-incubation ChE activities were compared to their respective initial activities. Samples found to have a significant increase in activity of at least 5% were considered exposed to a CA. All enzyme activities were measured in triplicate. A one-tailed Student's *t*-test was used to determine if ChE activities at 37EC were significantly (p <0.05) increased over activities at 1EC. All reagents were purchased from Sigma Chemical Company, St. Louis, MO.

Laboratory Assays

Brain tissue from two fish of each treatment were collected and assayed for AChE activity and reactivation. Fish had been collected and stored at -80NC until preparation for analysis. Brains were removed from frozen carcasses, diluted 100-fold and homogenized in a 0.1 M Trizma (7.4 pH) buffer solution. Homogenization was accomplished with a Wheaton grinding chamber and Teflon7 pestle (Curtin Matheson Scientific, Inc., Atlanta, GA). Samples were kept on wet ice during preparation and were then assayed immediately to determine initial total ChE activity using a method of Ellman et al. [21] modified for use on a UVMax kinetic microplate spectrophotometer (Molecular Devices, U.S.A.).

Levels of carbamate-inhibited ChE were determined using a dilution technique for promoting the reversibility of the carbamate-enzyme bond [23]. Assay concentration of the colorimetric agent 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) was 3.23×10^{-3} M. The substrate for enzyme action was acetylthiocholine iodide (AThCh). Substrate concentration was 1×10^{-3} M for all analyses. Samples were divided into two equal aliquots, one of which was incubated at 1NC on wet ice and the other at 37NC. All samples were analyzed after 4 hours incubation. All postincubation ChE activities were compared to their respective initial activities.

Levels of OP-inhibited ChE were determined by a chemical reactivation method [22,24] in conjunction with the modified Ellman method described above. Diluted samples were split into two aliquots and incubated for 20 minutes at 25NC in the absence and presence, respectively, of pyridine-2-aldoxime methochloride (2-PAM, 1×10^{-4} M). Organophosphates are displaced from ChE by 2-PAM, resulting in reactivation of the enzyme. A one-tailed Student's t-test was used to compare triplicate means for significant (p<O.05) reactivation of the 2-PAM treated aliquots.

Chemical Analyses

The extraction procedure was the same for both bendiocarb and chlorpyrifos. Water samples were passed through a 3M C_{18} Bakerbond J disks (3M Inc., St. Paul, MN) on a six place solid phase extraction manifold (Millipore Corp., Bedford, MA). The disks were pre-conditioned by passing 5 ml of ethyl acetate through and allowing to dry for 5 minutes. This was followed by the addition of 10 ml of methanol and 10 ml of distilled water. Not allowing the disk to dry, the water sample was then passed and the disks dried. The sample was eluted with two 10 ml aliquots of ethyl acetate. The subsequent sample volumes were concentrated by a constant flow of nitrogen using a Meyer N-Evap with a glass bead bath of 50NC (Organomation Assoc., Berlin, MA). Samples were brought to final volume (2 ml) with ethyl acetate (chlorpyrifos) or methanol (bendiocarb).

Chlorpyrifos concentrations were determined by a Hewlett Packard 5890 Series II Gas Chromato-graph with a DB-1 column (30 m x 0.50 mm I.D., 1.5 Fm coating, J & W Scientific), and a Hewlett Packard 7673 auto sampler. A flame photometric detector was utilized. A Hewlett Packard 1090 HPLC equipped with a post-column reaction module (PCK 5000, Pickering Laboratories) and a Hewlett Packard 1046A fluorescence detector was used to quantify levels of bendiocarb in the water samples. Cowles et al. (this report), provide the particular parameters set for the analysis of bendiocarb and chlorpyrifos.

RESULTS

Acute Toxicity Tests

The 96h tests were conducted twice with chlorpyrifos and three times with bendiocarb. The first tests with each pesticide failed because a lack of dose response. The second bendiocarb test failed because control mortality exceeded the acceptable level of 20%. The final tests also failed due to a chemical delivery problem. Mortality did not coincide with pesticide levels measured and the concentrations measured were not consistent with the nominal values. Total and partial kills were obtained but when plotted against the measured concentrations, a highly correlated dose-response was not evident.

Brain Cholinesterase Assays

The results of the *in situ* exposure to bendiocarb are presented elsewhere in this report (Cowles et al.). The fish taken from the acute laboratory exposures to chlorpyrifos and bendiocarb did exhibit a cholinergic response. All chlorpyrifos exposed fish sampled had depressed brain AChE levels. The percent AChE reactivation of these fish is expressed graphically in Figure 1. Laboratory fish appeared to be more sensitive than wild fish from the Ocean Course. Those exposed to the highest nominal concentration exhibited reactivation greater than 600%.

Bendiocarb-induced depression of AChE in the brain of sheepshead minnows was not as substantial as that produced by chlorpyrifos. No fish sampled exhibited more than 100% reactivation. The correlation of reactivation with nominal concentration of bendiocarb was poor ($r^2<0.250$) (Figure 2). No significant difference was seen between laboratory fish and those collected from the lagoon system on the course. The small correlation coefficient could be attributed to an apparent threshold effect near 500 Fg/l (nominal). At concentrations greater than this the depression does not seem to increase.

DISCUSSION

The failure of the acute toxicity tests was unfortunate, but with the data already available and the fate characteristics discussed elsewhere in this report acutely toxic exposure to these two pesticides on the Ocean Course, Kiawah Is., SC is very unlikely. However, we have demonstrated that sublethal exposure can lead to significant acetylcholinesterase depression, even with a small sample size. The ecological relevance of this is unknown. Behavior of the fish may be altered in a manner that potentially increases the risk of predation and thus foodchain transfer of the pesticides. Sublethal exposure to pesticides in concert with drastic fluxuations of dissolved oxygen may prove deadly to these and other fishes of the lagoon system.

The ongoing acute toxicity testing and AChE dose-response assays will help firm up these hypotheses and possibly lead to a cost and time effective method for assessing the hazard of golf course chemicals to fish. At this point, I believe it is safe to say that the conservative chemical management practices on the Ocean Course have had little or no toxic effect on sheepshead minnows or mosquitofish. Some behavioral tests, as well as species distribution studies, should be conducted to quantify possible ecological relevance of a chronic sublethal exposure scenario.

ACKNOWLEDGEMENTS

I would like to take this opportunity to thank the following people for their generous help in field collection and laboratory work throughout this project: Frank Bailey, James Cowles, Vincent Leopold, and Thomas Rainwater. I extend special gratitude toward Tena B. Bailey for all of her efforts in keeping the supplies coming and the rental vehicles available. Finally, I thank the United States Golf Association and the South Carolina Coastal Council for the financial support that made this research possible.

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Table 1. Acute toxicities of chemicals used or found on the Ocean Course.

Xenobiotic	LC5O Range
Acephate	100->I,OOO mg/l
Bendiocarb	0.4-1.5 Fg/l
Chlorpyrifos	2.4--280 Fg/l
Glyphosate	"Not Toxic"
MSMA	"Low Toxicity"
PCP	0.22 mg/l

Table 2. Fish species found in and around the lagoon system of the Ocean Course.

Common Name	Scientific Name	Where Collected
Carp	unknown	Front Nine
Mosquitofish	*Gambusia affinis*	Ubiquitous
Mummichog	*Fundulus heteroclitus*	Front Nine
Sailfin Molly	*Poecilia latipinna*	Ubiquitous
Sheepshead Minnow	*Cyprinodon variegatus*	Ubiquitous
Inland Silverside	*Menidia beryllina*	Ubiquitous
Striped Mullet	*Mugil cephalus*	Ubiquitous

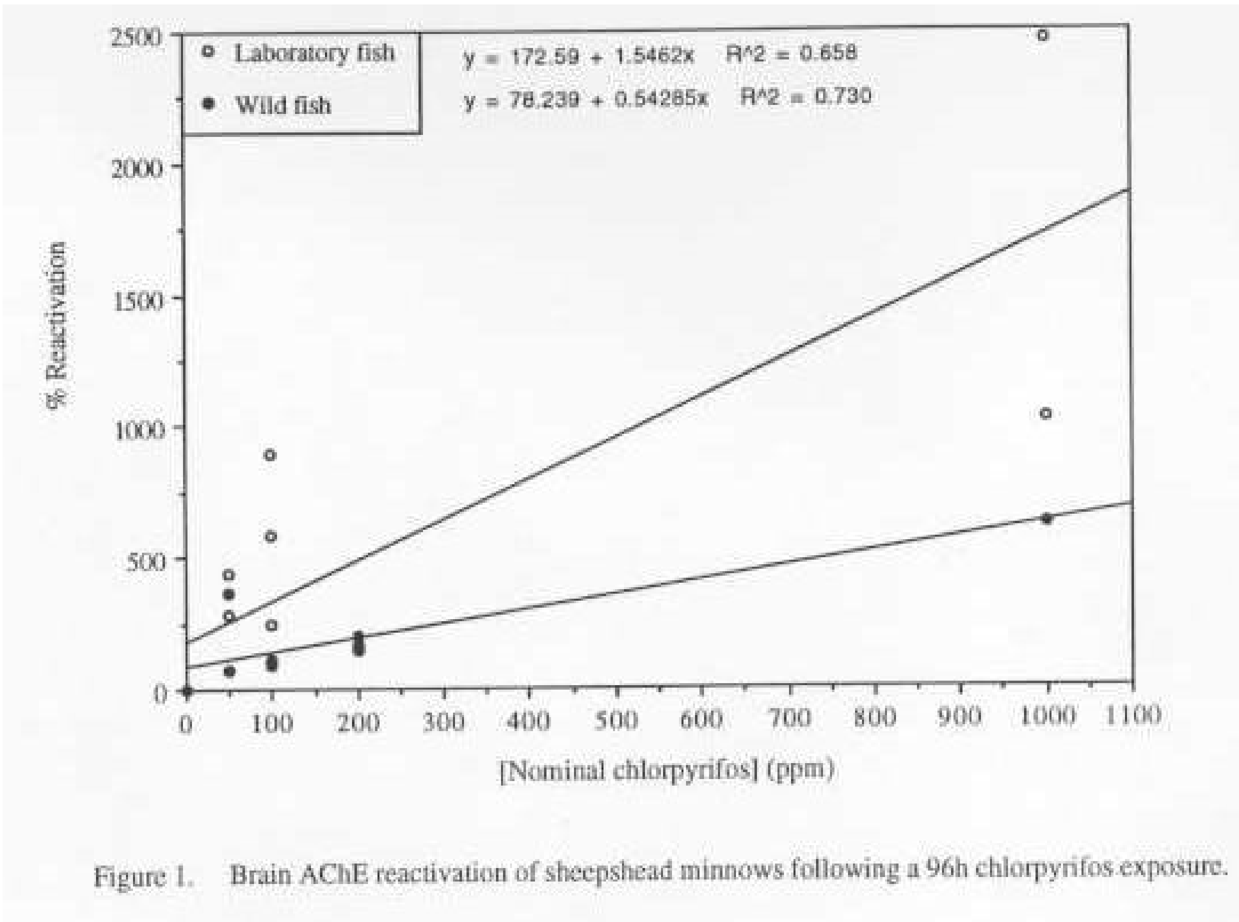

Figure 1. Brain AChE reactivation of sheepshead minnows following a 96h chlorpyrifos exposure

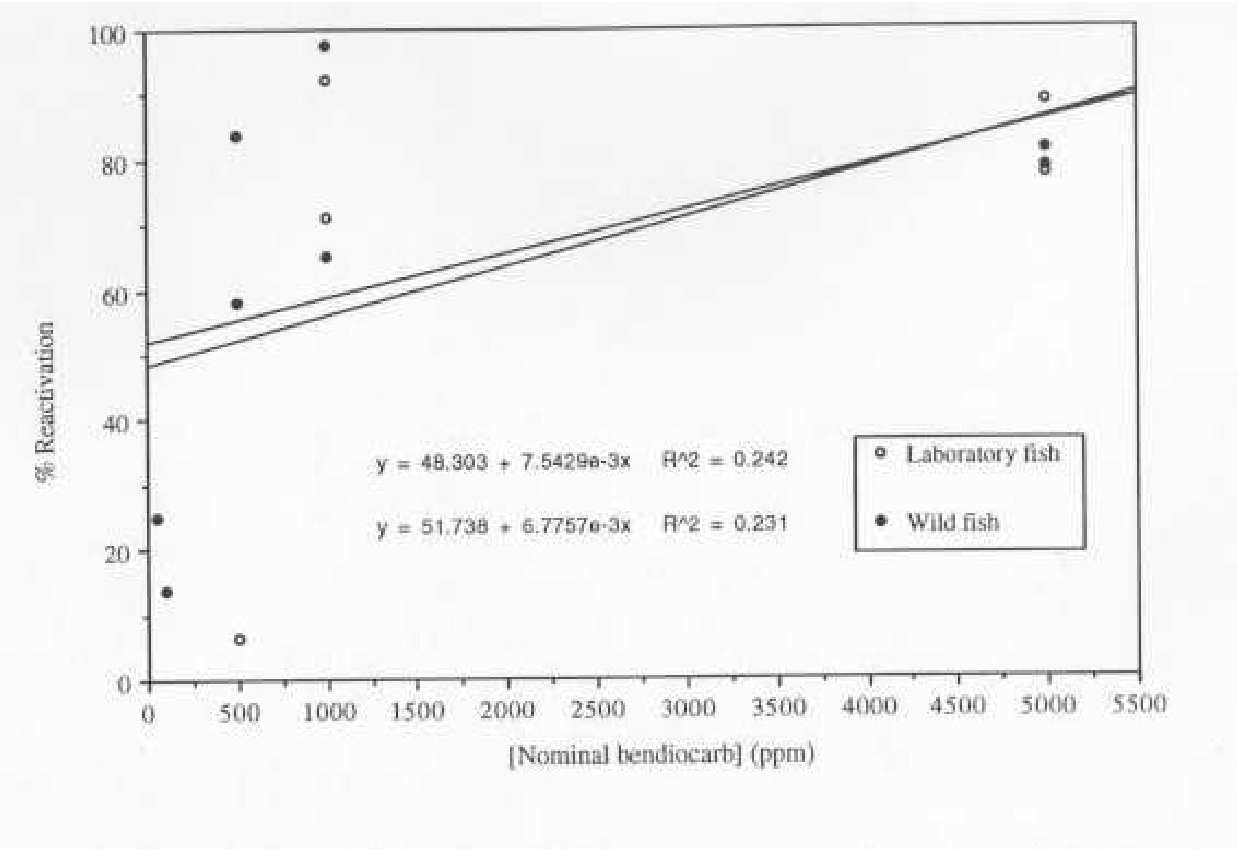

Figure 2. Brain AChE reactivation of sheepshead minnows following a 96h bendiocarb exposure.

Figure 2. Brain AChE reactivation of sheepshead minnows following a 96h bendiocarb exposure

CHAPTER 7

DETERMINATION OF UPTAKE AND PEROXIDASE RESPONSE AS INDICATORS OF SUBLETHAL INSECTICIDE EXPOSURE TO MACROPHYTES

DETERMINATION OF UPTAKE AND PEROXIDASE RESPONSE AS INDICATORS OF SUBLETHAL INSECTICIDE EXPOSURE TO MACROPHYTES

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Abstract - Both aquatic and terrestrial plants were utilized to assess the impact of insecticides used on the Kiawah Island Ocean Course. The peroxidase (POD) response of *Ruppia maritima* (a rooted submersed macrophyte) to Fenamiphos, Chlorpyrifos, and Bendiocarb was tested as a possible biomarker of sublethal environmental exposure to insecticides. Dose-response tests were run in the laboratory for each of these chemicals. It was determined that POD activity in *R. maritima* did not respond in a dose-dependent manner to any of these compounds. Accumulation of insecticides was also measured in both turfgrass and *R. maritima* in conjunction with insecticide applications to the Ocean Course. Bendiocarb residues were found in turf 24 hours post application and returned to below detection in four days. Bendiocarb residues were found to be highest in aquatic plant tissue one day post-application.

Keywords - Aquatic plants *Ruppia maritima* Insecticide Accumulation

INTRODUCTION

Higher plants are often overlooked as part of ecotoxicological testing. Testing to assess effluent toxicity usually involves the fathead minnow, *Pimephales promelas*, the invertebrate, *Daphnia magna*, and the green alga, *Selenastrum capricornutum* [1]. These tests are designed to indicate the general environmental health of a system. They are not complete, however, because they do not include information on the health of the total ecosystem, which would include higher plants [2].

Aquatic and terrestrial higher plants are primary producers, and as such are components of a healthy ecosystem. It is a common misconception in ecotoxicity testing that water quality which is sufficient to protect algae is also sufficient to protect higher plants [2]. However, it has been demonstrated with heavy metals, herbicides, and effluents that there is poor agreement between test results with algae and higher plants [3,4]. These studies reemphasize the need for more testing with higher plants, not only to determine toxicity but also to find the ecosystem relevance of this toxicity.

The predominant aquatic macrophytes in the lagoons on the Kiawah Island Ocean Course are Cattails (*Typha sp.*) and widgeon grass (*Ruppia maritima*). *R. maritima* is a rooted submerged plant and Cattails are rooted emergent plants. We utilized *R. maritima* for aquatic plant work in this study, as it is a known waterfowl food [5] and could be involved with food chain effects of pesticides. Also, *R. maritima* is in culture in our laboratory, making preliminary laboratory studies more controllable.

Turf patches from the course were analyzed for insecticides, because the turf is utilized by certain waterfowl [6], leading to a possible route of nontarget pesticide exposure. We collected aquatic plants for residue analysis from Ocean Course lagoons in conjunction with insecticide applications to determine the mobility and bioavailability of these chemicals in the tile drain and lagoon system.

In many instances, acute toxic responses of organisms to low levels of insecticides are not seen. However, there may be underlying sublethal impacts, which can potentially cause long term shifts in ecosystem structure and/or function. These effects would not be noticed with conventional chemical analyses or acute bioassays.

In response to stress, the activity of certain enzymes increases in plants. These changes in enzyme activity are often seen at low levels of phytotoxicity, before visible symptoms are seen [7]. Peroxidase (POD) induction is a general response of higher plants to a variety of stressors, including metals [8,9,10], air pollutants [11], and herbicides [8], and has been utilized to evaluate potential phytoxicity of metal contaminated soil and sediment [8,9,10]. This technique has not been tested to determine phytotoxicity of insecticides, however. While the physiological role of POD induction is unclear, it is believed to be significant in scavenging H_2O_2 and organic peroxides, which are formed by plants in response to a variety of stress conditions [7].

To ascertain sublethal effects of insecticide exposure to aquatic plants on the course, we used the method of Byl [8] to measure the peroxidase activity. There was no attempt to coordinate sampling with herbicide applications on the course, as they were only spot sprayed in the sand dunes. We assumed that the herbicides were applied sparingly and did not reach the surrounding sensitive aquatic systems.

MATERIALS AND METHODS

We collected *R. maritima* plants from the lagoons on the Ocean Course following the application of insecticides and at other times throughout the study period. Plants were harvested and stored in ziploc bags on ice until placement in a laboratory freezer.

For pesticide residue analysis, aquatic plants were finely chopped with scalpels and mixed with 35ml 25:75 acetone/hexane and 2g anhydrous sodium sulfate (oven dried at 130°C for 24 hours) and placed on an orbital shaker set at 250 rpm, for 30 minutes. After shaking, the acetone/hexane was decanted through Whatman 41 filter paper containing 30g of anhydrous sodium sulfate pre-wetted with acetone/hexane. The sodium sulfate filter was rinsed three times with approximately 5ml acetone/hexane. The sample was then rotary evaporated to dryness using a Buchi RE 111 Cold Finger Rotavapor with a water bath set at 40°C. Samples were then diluted to final volume in methanol for analysis by high performance liquid chromatography (HPLC) or gas chromatography (GC).

To examine the utility of the peroxidase (POD) assay for the determination of sublethal impacts of insecticides to aquatic plants, we ran dose-response tests with chemicals which are used on the Ocean Course. Fenamiphos and Chlorpyrifos were chosen as representative organophosphate insecticides, and Bendiocarb as a carbamate insecticide. Plants were placed in 100ml test tubes in artificial seawater (15g/l salinity) for one week prior to testing in order to allow them to acclimate to the test conditions. After the acclimation period, we replaced the solutions in the test tubes with either artificial seawater for a control or the same containing a given concentration of one of the above chemicals (Table 1). The test period was seven days in a growth chamber at 25°C with a photoperiod of 16hrs light: 8hrs dark. At the end of the seven day period, the plants were harvested and analyzed for POD by the method of Byl [8].

With the cooperation of the Ocean Course managers, we carried out a monitored application of Bendiocarb 2.5 G to the #7 green on the course. The bendiocarb was applied to the green in a granular form at a target rate of 1.9 lb active ingredient/1000 ft^2. To simulate a "worst-case" scenario, the green was then irrigated sufficiently to cause surface runoff for approximately 20 minutes into the drain system. The outlet from the drain emptied into a nearby brackish lagoon. In this way, the maximum amount of chemical possible would be washed into the lagoon and the exposure and effects could be monitored. We carried out bendiocarb residue analysis on *R. maritima* from the output lagoon and turf patches from the dosed green pre- and post-application.

RESULTS AND DISCUSSION

Chemical analysis of *R. maritima* samples taken in conjunction with insecticide applications (chlorpyrifos, fenamiphos, bendiocarb) to the Ocean Course showed only detectable levels of bendiocarb (Fig. 1). Bendiocarb residues are highest immediately after application and decrease over time. Since *R. maritima* is an aquatic plant, this data indicates bendiocarb does reach the lagoons on the course, leading to possible foodchain uptake of the chemical. However, since the bendiocarb LD50 value for mallards is 3.1mg/kg [12], an average mallard weighing approximately 1 kg [13] would have to eat 10,000g of plant material to reach an acutely toxic dose. This makes ecological relevance of exposure to *R. maritima* undetermined. Other chemicals applied are either not accumulated by *R. maritima* or they are not reaching the lagoons in significant amounts.

The POD dose-response tests showed that *R. maritima* did not respond in a dose-dependent manner to any of the chemicals tested. These results indicate that the POD response of *R. maritima* to these insecticides is not a useful environmental indicator. However, the use of POD as an indicator of nontarget exposure to herbicides is not ruled out by this study. Herbicides were not included in this study, but Byl [8] showed a POD dose-response to an herbicide with *Hydrilla verticillata*.

It seems that on the Ocean Course plant ecotoxicity studies involving the overall water quality may be more appropriate than biochemical studies. The water quality of the nutrient-rich system may be impacting the habitat requirements of the aquatic plants more than any specific chemical. It was shown by Dennison et al. [14] in Chesapeake Bay that submerged aquatic vegetation can be used to assess water quality. They used habitat requirements of the plants to characterize the water quality of the Bay.

The authors established quantitative levels of minimum relevant water quality parameters to support submersed aquatic vegetation, such as total suspended solids, chlorophyll *a*, dissolved inorganic nitrogen, dissolved inorganic phosphorus, and light attenuation coefficient. Each of these parameters has an impact on the amount and depth of light penetration in an aquatic system. They found that a slight deviation from the minimum requirements of any of these parameters could lead to the disappearance of the submersed aquatic plants.

From the turbidity, algal biomass, nutrient loading (see Fouts et al., this report) and lack of *R. maritima* in some of the lagoons on the Ocean Course, it appears that a similar study should be carried out in these lagoons. Lagoons located off of the Ocean Course could be used as reference sites. By using aquatic plants as living "light meters", a link can be made between water quality and the "health" of vegetation. If poor water quality is eliminating aquatic plants, such as *R. maritima*, from certain lagoons then a part of the food chain has been removed and vital habitat and cover for invertebrates and fish has been lost. Both of these consequences indicate a disturbance to the environmental health of the system.

After the monitored application of Bendiocarb to the seventh green, the chemical was found in the turf at 24 hours and was seen to decline to below detection limits over 4 days (Figure 2). A slight increase in bendiocarb was seen in *R. maritima* from the outlet lagoon at the 2 hour timepoint, but not any of the others. Water samples from the lagoon showed no detectable bendiocarb.

This relatively brief and low dose of bendiocarb to the lagoon appears to leave a very small window of time when possible toxicity could occur in the aquatic systems on the course. And, since this was a worst case scenario, it is quite possible that under normal conditions with no surface runoff even less chemical reaches the lagoon system. In fact, soil column studies (Cowles et al., this report) designed to mimic the conditions on Ocean Course greens showed no movement of bendiocarb past 10 cm into the column over a 5 day period.

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Fenamiphos	Chlorpyrifos	Bendiocarb
0.1 mg/l	0.01 mg/l	0.01 mg/l
1.0 mg/l	0.03 mg/l	0.03 mg/l
10 mg/l	0.1 mg/l	0.1 mg/l
100 mg/l	0.3 mg/l	0.3 mg/l
------------------	------------------	1.0 mg/l

Table 1. Range of chemical concentrations tested in POD assays with *R. maritima*.

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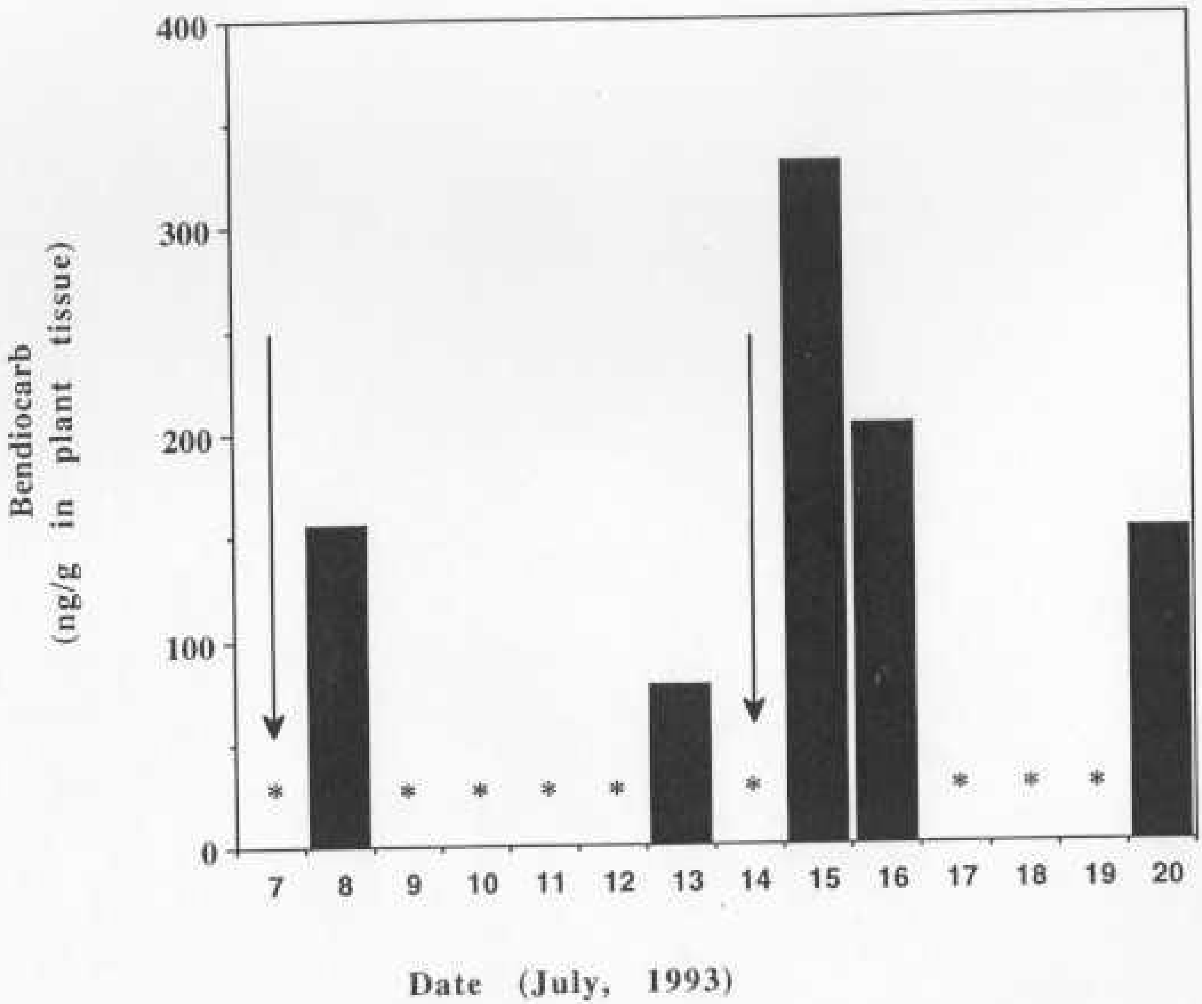

Figure 1. Bendiocarb in Ruppia maritima from Ocean Course lagoons. Arrows represent bendiocarb application dates. *No samples collected.

Figure 1. Bendiocarb in Ruppia maritima from Ocean Course lagoons. Arrows represent Bendiocarb application dates. *No samples taken.

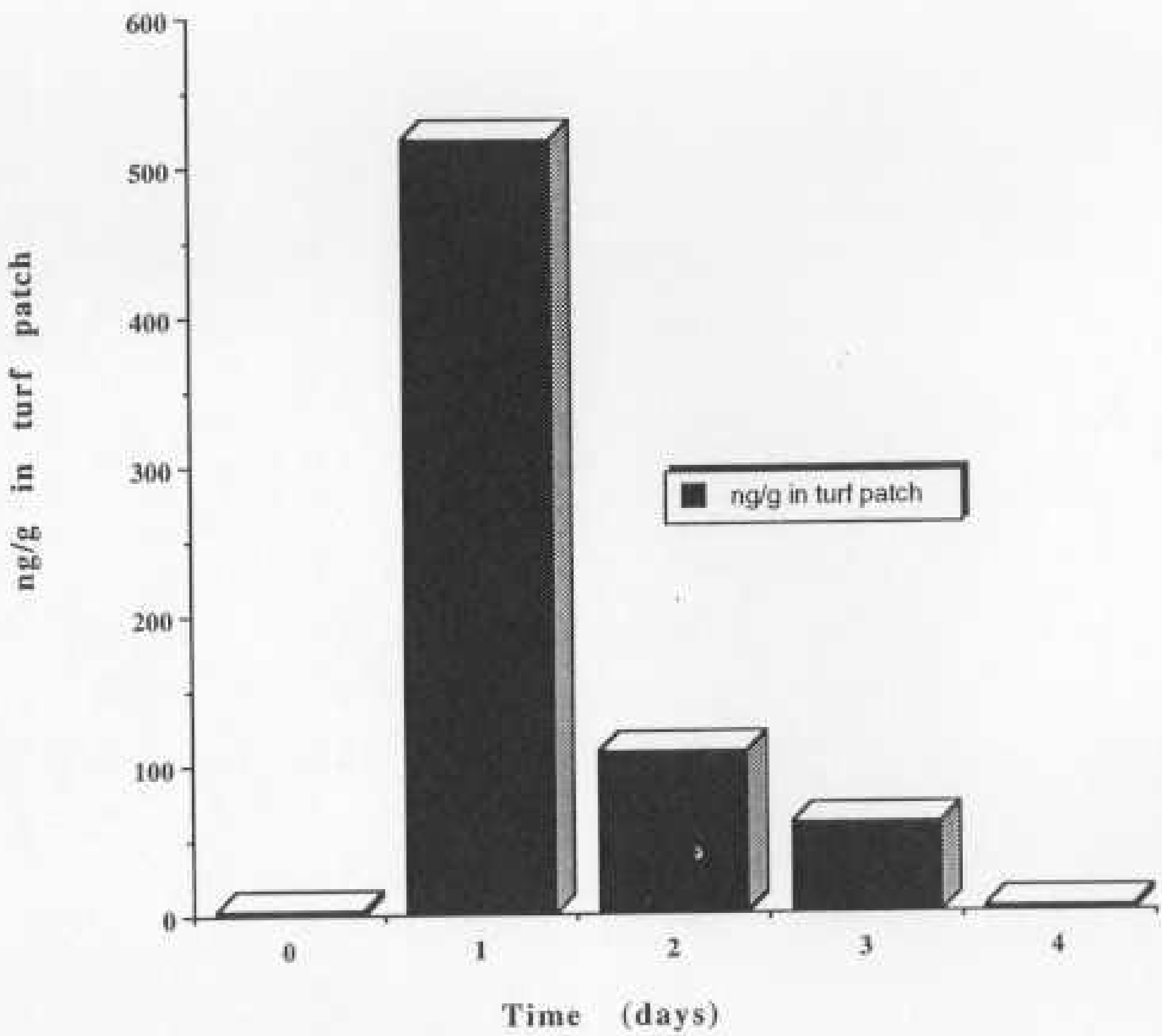

Figure 2. Bendiocarb in turf after August 24, 1993 application to green #7.

Figure 2. Bendiocarb in turf from the planned application on green number 7

CHAPTER 8

AN ECOLOGICAL RISK APPROACH TO THE EFFECTS OF NUTRIENT LOADING ON A SOUTH CAROLINA GOLF COURSE

AN ECOLOGICAL RISK APPROACH TO THE EFFECTS OF NUTRIENT LOADING ON A SOUTH CAROLINA GOLF COURSE

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Abstract-Intensive fertilizer application to golf course turfgrass often generates high nutrient levels in golf course lagoons and adjacent wetlands as runoff enters the aquatic system. Productivity of algae and other planktonic organisms increases greatly in response to nutrient availability and excessive growth may result in adverse conditions. Construction of the irrigation water recapture system on the Ocean Course, Kiawah Island, South Carolina, provided us with an opportunity to track golf course runoff to contained lagoon systems and adjacent wetlands. The water recapture system serves to minimize nutrient loading into adjoining brackish marshes.

Chlorophyll *a* concentrations in two lagoons were measured spectrophotometrically to assess changes in primary productivity throughout the year. Levels of chlorophyll *a* over 1000 mg/m^3 and biomass over 110 g/m^3 were measured, indicating a highly productive system. Nutrient analyses for nitrate and phosphate indicate levels as high as 21 ppm and 3.8 ppm, respectively, and provide evidence of the potential for off-site eutrophication. Nutrient fluxes and algal productivity are addressed in terms of seasonal variation in irrigation practices, storm events and water quality parameters.

Keywords-Eutrophication golf course wetlands

INTRODUCTION

Intense fertilizer application to golf turfgrass often generates high nutrient levels in golf course lagoons, as runoff enters aquatic systems. Algal productivity increases greatly in response to nutrient availability. Excessive eutrophication results in adverse effects [1]. Algae have the capability to add tremendous amounts of organic matter to lentic systems [2]. Hypertrophic systems may become anoxic as dense algal mats prevent oxygen from dissolving into the water's surface. Reductions in dissolved oxygen can provoke "fish kills" over large areas. Extensive phytoplankton growth may generate offensive odors as anaerobic decomposition occurs.

Seasonal application of chemicals (fertilizers and pesticides) to the golf course, and subsequent runoff, may generate a multiplicity of effects within the aquatic community. Management of nuisance algal populations often involves algicides and biological controls. Because phytoplankton form the foundation of aquatic food chains, alteration of the algal community may have repercussions on higher trophic levels.

Construction of the irrigation water recapture system on the Ocean Course, Kiawah Island, provided us opportunity to track golf course runoff to contained lagoon systems and to examine the effects of turf management practices on the aquatic organisms. Two Ocean Course lagoons were chosen for analysis during the 1992-1993 field season. The Back Nine lagoon receives recaptured water from the 13th, 14th, 15th and 16th fairways, greens and tee boxes (Fig. 1a). This water may contain chemicals and nutrients from the soil or surface runoff from approximately 16 acres. The Recycling Lagoon is the second study site. This lagoon contains water recaptured from each of the back nine holes of the golf course. Cumulative chemical loading from diverse management techniques may be detectable at this point.

Concentration of the photosynthetic pigment chlorophyll *a* is commonly used as an index of primary productivity [3]. Biomass or standing crop is a quantitative estimate of the mass of live organisms within a given area or volume and is most accurately generated by measuring ash free dry mass. Chlorophyll *a* comprises about 1-2% of the dry weight of planktonic algae. These two measurements will be used to assess phytoplankton productivity in the Ocean Course lagoons.

Phytoplankton biomass is related to the amount of nitrogen and phosphorus available and the distribution of these nutrients within the aquatic system. Nitrate (NO_3^-) and orthophosphate (as HPO_4^{2-}) concentrations were measured at points of lagoon inflow to estimate nutrient input.

1993 OBJECTIVES

- Estimate primary productivity in the Ocean Course lagoons using measures of chlorophyll *a* (chl*a*) and ash free dry mass (AFDM).
- Quantify nitrate and phosphate levels at lagoon inflows and relate these to primary productivity.
- Trace seasonal changes in lagoon primary production as estimated using chl*a*.
- Correlate standing crop of phytoplankton and chl*a* with nutrient levels emanating from fertilizer application and the use of secondarily-treated effluent used for irrigation.

MATERIALS AND METHODS

Field Component

Samples for nutrient analyses were taken from water flowing into the Back Nine, Recycling and Effluent lagoons and Willet Pond, approximately every three weeks. Water was collected in 50 ml centrifuge tubes and placed on ice for transfer to the laboratory. Samples were either frozen or filtered and analyzed within 24 hours.

On each lagoon, three transects representing roughly one-third of the surface area were sampled. Each transect had three evenly-spaced sampling stations (Figure 1b). Samples were collected approximately every three weeks at each of the 18 sampling stations from an aluminum canoe. Water quality (dissolved oxygen, pH, temperature, conductivity, and redox potential) was measured at depths of 0.3 m, 1.0 m and then every 0.5 m until reaching the bottom, using a Hydrolab Surveyor 3 Water Quality Logging System (Hydrolab Corp., Austin, TX). Readings were electronically stored in the Hydrolab and simultaneously recorded by hand on waterproof data sheets. Water samples for chl*a* and AFDM analyses were collected using a one-liter Kemmerer bottle. Samples were collected 50 cm below the surface and transferred to 1 quart Cubitainers**7**. The containers were kept in the dark at 4EC for transport to the laboratory.

Nutrient Analyses

Analyses for nitrate (NO_3^-) and orthophosphate (as HPO_4^{2-}) were performed using ion chromatography (IC). The method involves modifications of Waters method #A-102 [4]. Approximately 2 ml of sample water is filtered through a 0.45 ìm syringe filter directly into a 2 ml autosampler vial (Chromatography Research Supplies, Addison, IL). The water is injected into the IC system without further treatment. The system consists of a Waters 510 dual head HPLC pump, a Waters Intelligent Sample Processor, or WISP (autosampler/injector), a Waters 431 Conductivity Detector, and a Waters 431 Data Module (integrator) (Millipore Corp., Milford, Mass).

The original method requires a single column. Instead, we used two columns in series to separate the analytes of interest from other salts in the brackish water. The first column used is a Waters IC-Pak**J** Anion HC (high capacity) anion exchange column (1.6 x 150 mm, 10 ìm particle size). The second column in series is an IC-Pak**J** Anion HR (high resolution) anion exchange column (4.6 x 75 mm, 6 ìm particle size). The eluent (aqueous 0.01 M borate/gluconate buffer containing 12% (v/v) acetonitrile) is the same as that given in Heckenberg et al. [4] except n-butanol is omitted. Eluent flow rate is 0.9 ml/min (isocratic). Sample injection volume is 100 ìl. For sample anion quantitation, sample peak heights are compared to standard curves in an external standard quantitation procedure. Standard concentrations range from 0.2 to 20 ppm.

Water Quality

Once water quality data were collected and the Hydrolab returned to the laboratory, the information was downloaded to a personal computer. Data were stored in a spreadsheet program, allowing statistical and graphical manipulation.

Phytoplankton Productivity Analyses

In the laboratory, samples were filtered using Millipore**7** apparatus, a vacuum pump and glass fiber filters (Gelman AE; 1 ìm porosity). Chlorophyll *a* was extracted by placing the filter in a vial and treating with 15 ml of 90% aqueous acetone (10% saturated magnesium carbonate solution). Samples were then extracted for 24 hours at 4EC in the dark.

Chlorophyll *a* concentration was determined spectrophotometrically on a Beckman DU-70 spectrophotometer. Optical densities at 664 and 750 nm were measured and recorded for each sample. Following this reading, each sample was acidified using 100 µl of 0.1 *N* HCl and absorbance measured at 665 and 750 nm to correct for absorbance by the pigment pheophytin.

Corrected values for chlorophyll *a* (OD 664 - OD 750_{before} = 664_b and OD 665 - 750_{after} = 665_a) per cubic meter were determined using the following:

$$\text{Chlorophyll } a\text{, mg/m}^3 = \frac{26.7(664_b - 665_a) \times V_1}{V_2 \times L}$$

where:

V_1 = volume of extract, L,

V_2 = volume of sample, m^3,

L = light path length or width of cuvette, cm, and

664_b, 665_a = optical densities of 90% acetone extract before and after acidification, respectively.

The value 26.7 is the absorbance correction, *A X K*, where *A* is the absorbance coefficient of chlorophyll *a* at 664 nm = 11.0 and *K* is the ratio correcting for acidification [5].

Extracts and filters were then transferred to porcelain crucibles and placed under a hood until the acetone had evaporated. The crucibles and contents were dried at 105°C for 24 hours and dry weight measured and recorded. Samples were ashed in a muffle furnace at 500°C for one hour. Ash free dry mass was calculated and recorded.

Phytoplankton community composition was determined by examining water samples from each transect during each sampling period using an inverted microscope (300X).

RESULTS AND DISCUSSION

Field Component

Water samples for primary productivity and hydrological measurements were taken 22 times during the 1992-1993 field season. Nutrient samples were collected 12 of those sampling periods. Transect A, on the Back Nine Lagoon, and Transect D, on the Recycling Lagoon, were chosen to examine the relationship between nutrient input and chlorophyll *a* biomass, as these two transects were closest to the point of nutrient sample collection (Fig. 1b).

Nutrient Analyses

Chromatographic column retention times for nitrate and orthophosphate were approximately 30.7 and 35.7 minutes, respectively (Fig. 2). Because these elute significantly after chloride (Cl^-) (RT =

13.7 min) and well before sulfate (SO_4^{2-}) (RT = 55.0 min), nitrate and orthophosphate could be quantified even in the presence of large chloride and sulfate concentrations, such as those found in brackish water surrounding the course. Unsatisfactory results were obtained using either column alone because high concentrations of chloride and sulfate grossly overloaded the columns and rendered them ineffective in separating these "interferences" from nitrate and orthophosphate.

Detection limits for nitrate were approximately 0.1 ppm and for orthophosphate approximately 0.5 ppm. These are higher than usual detection limits in a single column system due to the band-broadening from the longer in-column time. Nitrite (NO_2^-) would have been another useful species to monitor but the peak was often encompassed by the chloride peak and thus measurement of nitrite was usually not reliable.

Orthophosphate concentrations in the lagoons averaged 2 mg/l (Appendix 1). Seasonal variation for both nitrate and orthophosphate peaked during the summer months, reflecting intensified irrigation and fertilizer application practices (Fig 3). Nitrate concentrations were up to five times greater in the Back Nine lagoon than in the Recycling lagoon, possibly due to more direct collection of drainage into the Back Nine lagoon.

Water Quality

In general, water quality parameters of the Recycling and Back Nine lagoons were similar throughout the year (Appendix 2.). Temperature, pH, and dissolved oxygen (DO) data from transect B site 5 and transect E site 14 (the middle site on the Back Nine and Recycling lagoons, respectively) were plotted as representative of the two lagoons (Figs. 4,5, and 6). These figures depict annual trends in lagoon physical characteristics between 07/25/92 and 08/08/93. Variation in water quality within transects and between transects was minimal for a given depth during any sampling event. Thus, conditions were fairly uniform across each lagoon.

During the warmer months the lagoons exhibited vertical stratification for all three parameters presented. This is most obvious in the temperature profiles, where readings taken during the warmer periods range widely, but are tightly clumped during the winter months (Fig. 4). Seasonal stratification of this type is typical of eutrophic systems [6].

Carbon dioxide consumption by photosynthetic phytoplankton raised pH in the zone of light penetration during the warmer months (Fig. 5). Removal of CO_2 from the water shifts the carbonate equilibrium [$CO_2 + H_2O$: H_2CO_3 : $H^+ + HCO_3^-$] to the left, increasing pH in surface waters, relative to that of deeper water. This may indicate a poorly buffered system.

Horizontal stratification of dissolved oxygen is also probably due to increases in algal productivity during warmer periods (Fig. 6). As temperature increases, photosynthetic activity is elevated in the surface strata resulting in high levels of dissolved oxygen. Decomposition of organic matter by bacteria decreases oxygen levels near the bottom. Such DO phenomena are indicative of an extremely productive system. Fertilizers in the runoff from the course are a possible contributing factor to this productivity. Severe oxygen depletions may occur at night in eutrophic systems because the algae, while not photosynthetically active, will continue to respire and consume O_2. This can be a problem even in near surface waters, resulting in the death of fish and other aquatic organisms [6]. We did not measure DO in the water at night, so it is not known if the lagoons exhibit this trait or not. The DO stratification, as with the other parameters, is much less pronounced in the winter months. This is due to reductions in both photosynthesis and respiration caused by the colder water temperatures and low light levels, and to the increased mixing in the somewhat isothermal water column.

Phytoplankton Productivity Analyses

Various criteria exist to describe the quality of freshwater systems. Generally, these standards are applied to reservoirs and lakes, but they are also useful for less resilient, smaller systems. According to a widely-accepted trophic state threshold value for chlorophyll *a* concentrations, those above 8.7 mg/m^3 are considered eutrophic [7]. Levels measured in the two lagoons studied generally exceed 100 mg/m^3, except those taken during February and early March, which still exceed the threshold value given (Figs. 7 and 8) (Appendix 3). With regards to mean yearly chlorophyll concentrations and seasonal maxima, the lagoons are characterized as strongly hypertrophic [8].

Organic biomass concentration, AFDM, was also high in both lagoons, averaging 30-40 mg/m^3 through the year and exceeding 120+ mg/m^3 during summer peaks.

A trophic index, chlorophyll *a* per AFDM estimates the level of autotrophic biomass relative to total plankton biomass [9]. The trophic index does not measure a specific community function, but rather characterizes the photosynthetic efficiency of the community. The hypertrophic state of the lagoons, manifested by high organic biomass, turbidity, and suspended solids, resulted in low photosynthetic efficiency in the lagoons studied (Fig 9). The limited autotrophic biomass, relative to total organic biomass, may also be a function the high nutrient loads inducing maximal phytoplankton biomass.

Chlorophyll *a* was only weakly correlated to nitrate and phosphate concentrations (Fig. 10 and 11). Phytoplankton growth rate is a function not only of nutrient availability, but of organism physiology as well. Sites of uptake, on the cell membrane, are limited and although uptake increases with availability, sites will become saturated at higher nutrient concentrations. Thus, uptake by the cells will eventually reach a maxima and remain stable even if nutrient input increases. Rate of uptake is also controlled by the amount of nutrient stored internally by the cell. This internal cell content may control the rate of population growth [10].

The dominant plankton observed during each sampling period was a cyanobacteria genera, *Anacystis*. This blue-green alga is common to hypertrophic systems [11]. Only rarely were other phytoplankton taxa encountered. This is not surprising since established populations of cyanobacteria may actually alter their surroundings, physically, chemically, and biologically in ways which favor the persistence of the species [12].

A major public concern with the proliferation of golf courses in environmentally sensitive areas (such as salt marshes or freshwater wetlands) is the potential for chemical and environmental violation of aquatic ecosystems "downstream" from the course. This includes pesticide contamination, sedimentation, or eutrophication. To-date our study indicates no measurable pesticide runoff into lagoons has occurred from application of pest control chemicals used on the Ocean Course.

However, because the Back Nine and Recycling lagoons on the Ocean Course receive irrigation and fertilization runoff from the golf course "watersheds" (greens, fairways, tee-boxes, etc.), the lagoon inflows contain sufficient nutrient concentrations to maintain an organically-rich aqueous medium. With respect to nitrates, phosphates, suspended solids and turbidity, the conditions are similar to secondarily-treated organic wastewaters. The hypertrophic conditions in the lagoons reflect the high nitrogen and phosphorus content of fertilizers used on turfgrass. Further, allochthonous organic material from sedges and cattails in the adjacent marshes contribute to the organic matter concentrations in the lagoons.

Low trophic index values, such as we measured in the Back Nine and Recycling lagoons (Fig. 9), are associated with hypertrophic conditions. We suspect turbidity in the lagoon limits photosynthesis to the upper few decimeters. Further, we expect that much planktonic biomass in the lagoons stems from heterotrophic activities of blue-green algae, dominant year-round in these systems.

Composition of the phytoplankton community is regulated by temperature and light. Typically, seasonal succession of phytoplankton in mesotrophic, temperate, freshwater systems occurs in this fashion: diatoms dominate in the spring, green algae in the summer, blue-green algae in the late summer and possibly diatoms in the fall. However, as Smith [13] has demonstrated for reservoirs, as phosphorus content, hence eutrophication and algal biomass, increases, there is a tendency for blue-green algae to dominate throughout the growing season. Unfortunately, excessive blue-green algae are detrimental to the immediate stability of the ecosystem. Their filamentous and colonial habit restricts grazing by filter-feeding zooplankton and reduces the system's trophic efficiency. The same toxins produced by nuisance blue-green algae to eliminate competitive species can also be toxic to zooplankton when algal blooms are dense [14]. Blue-green algae are buoyant and tend to float in unsightly clumps, forming scums on the surface. They often produce offensive odors, especially during blooms.

Nutrient concentrations in the Ocean Course lagoons indicate the movement of nutrient-rich runoff into the lagoons, and ultimately, into Willet Pond. With our focus during 1992-93 on the runoff from greens and fairways, we have demonstrated the potential for eutrophication to occur in Willet Pond. Constraints on time and effort precluded our investigation of water quality in Willet Pond during 1992-93. However, we received no reports of adverse effects within the pond. Hence, we suspect the lagoons are acting as "bio-reactors," limiting the movement of nutrients into the natural pond and estuary.

However, two critical questions remain: (1) flow retention time in the lagoons and (2) nutrient fate in the marsh channel leading to Willet Pond. The extensive emergent and submerged vegetation (living and dead) may provide a "sink" for nitrogen and phosphorus in golf course effluent. During this coming year, our investigation will focus on the fate of the effluent leaving the golf course.

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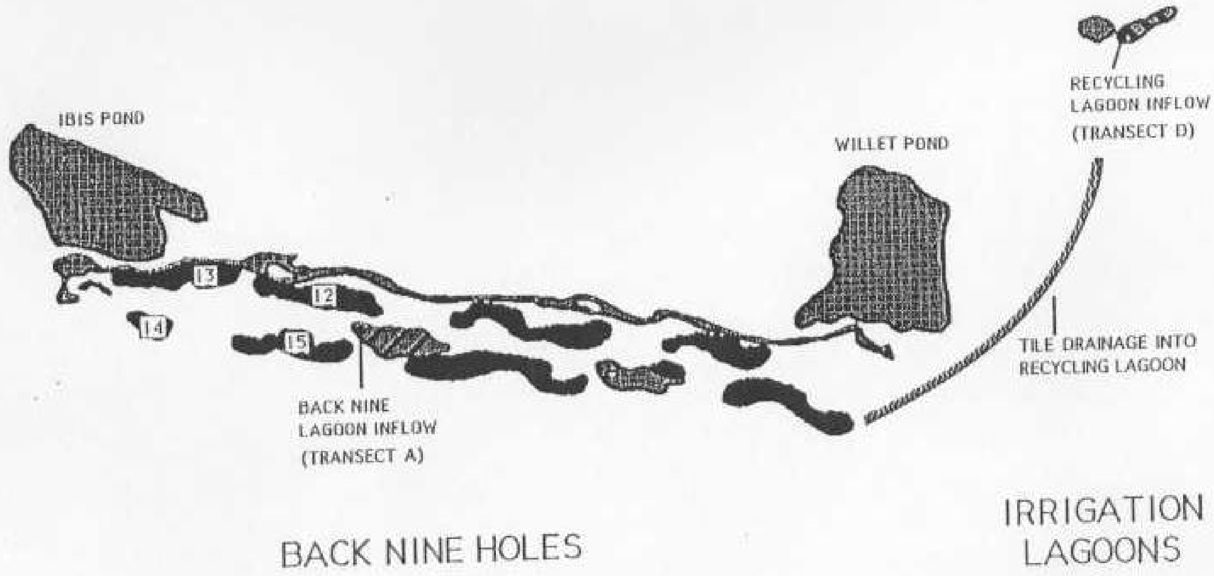

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Figure 1a. Study site for the 1992-1993 field season. The back nine holes and the irrigation lagoons, the Ocean Course, Kiawah Island, SC.

Figure 1a. Study site for the 1992-1993 field season. The back nine holes and the irrigation lagoons, the Ocean Course, Kiawah Island, SC.

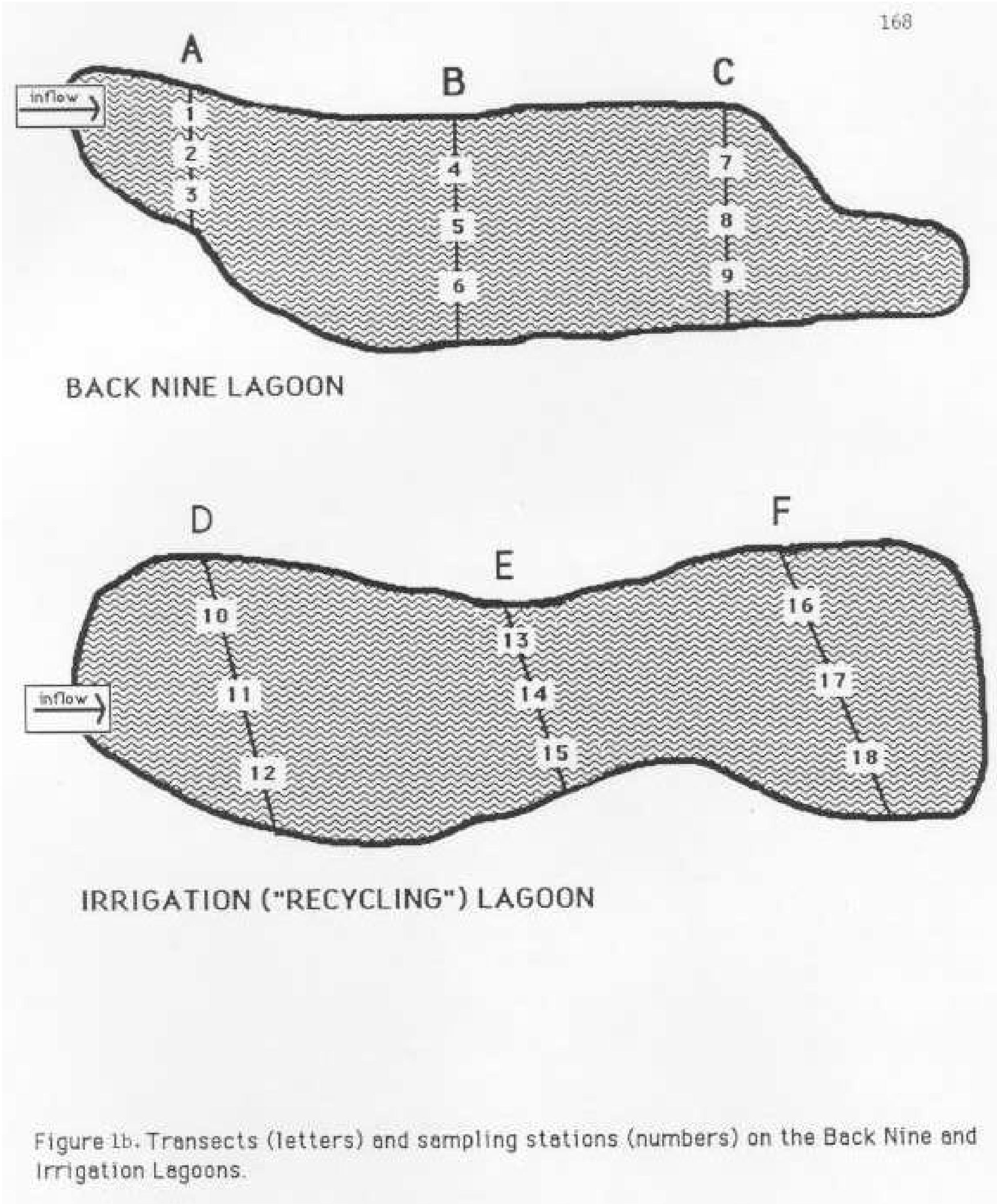

Figure 1b. Transects and sampling stations on the back nine and irrigation lagoons.

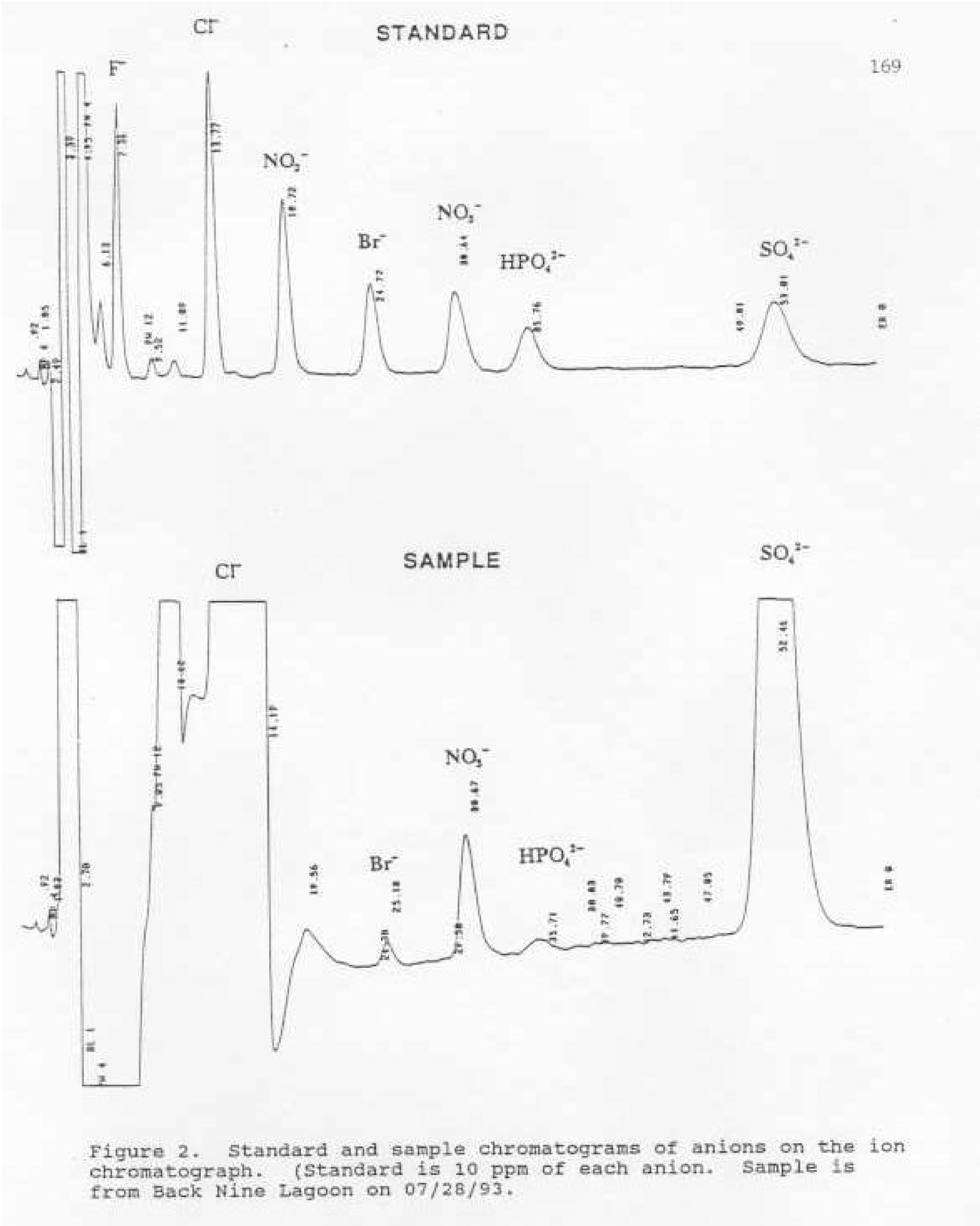

Figure 2. Standard and sample chromatograms of anions on the ion chromatograph.

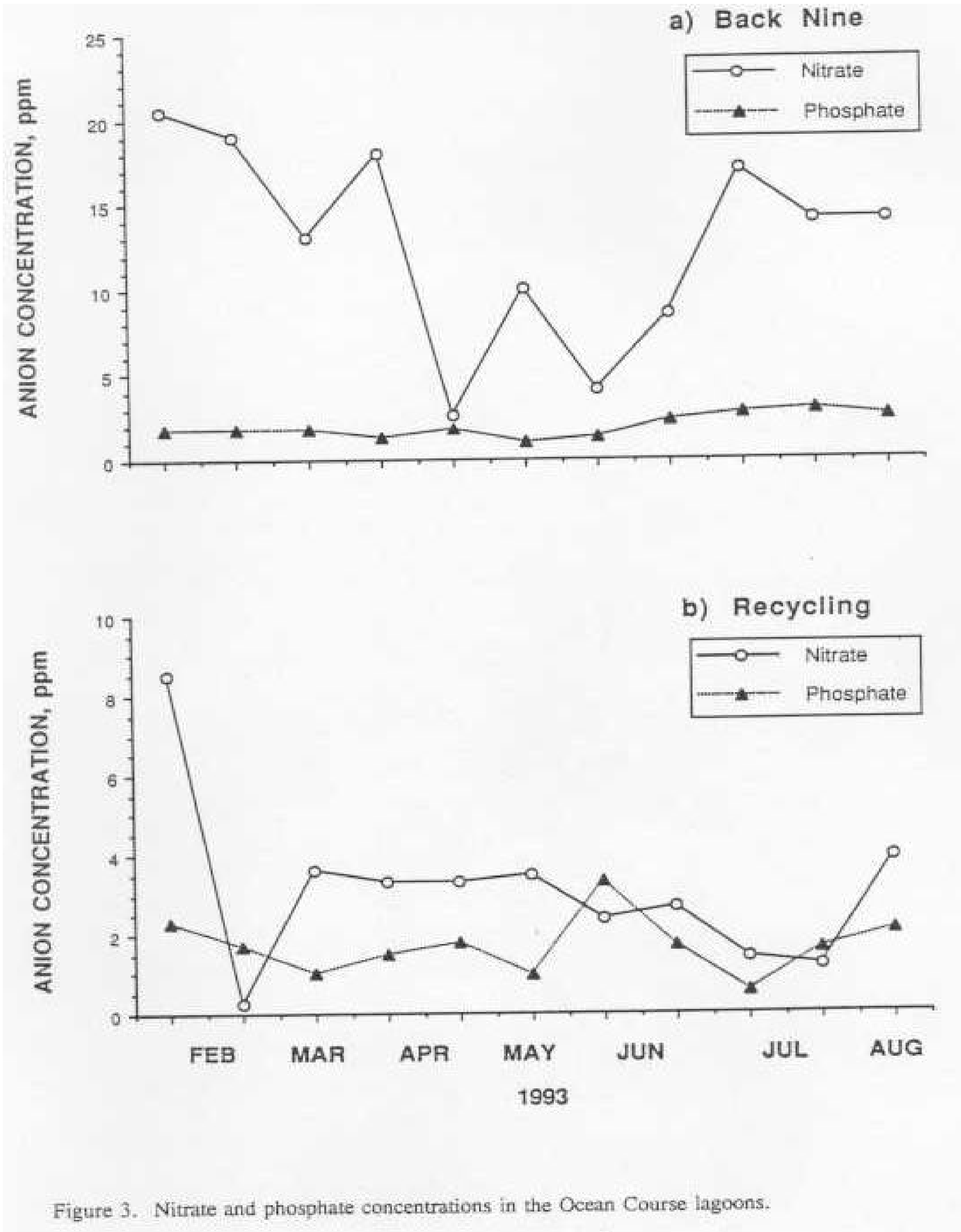

Figure 3. Nitrate and phosphate concentrations in the Ocean Course lagoons.

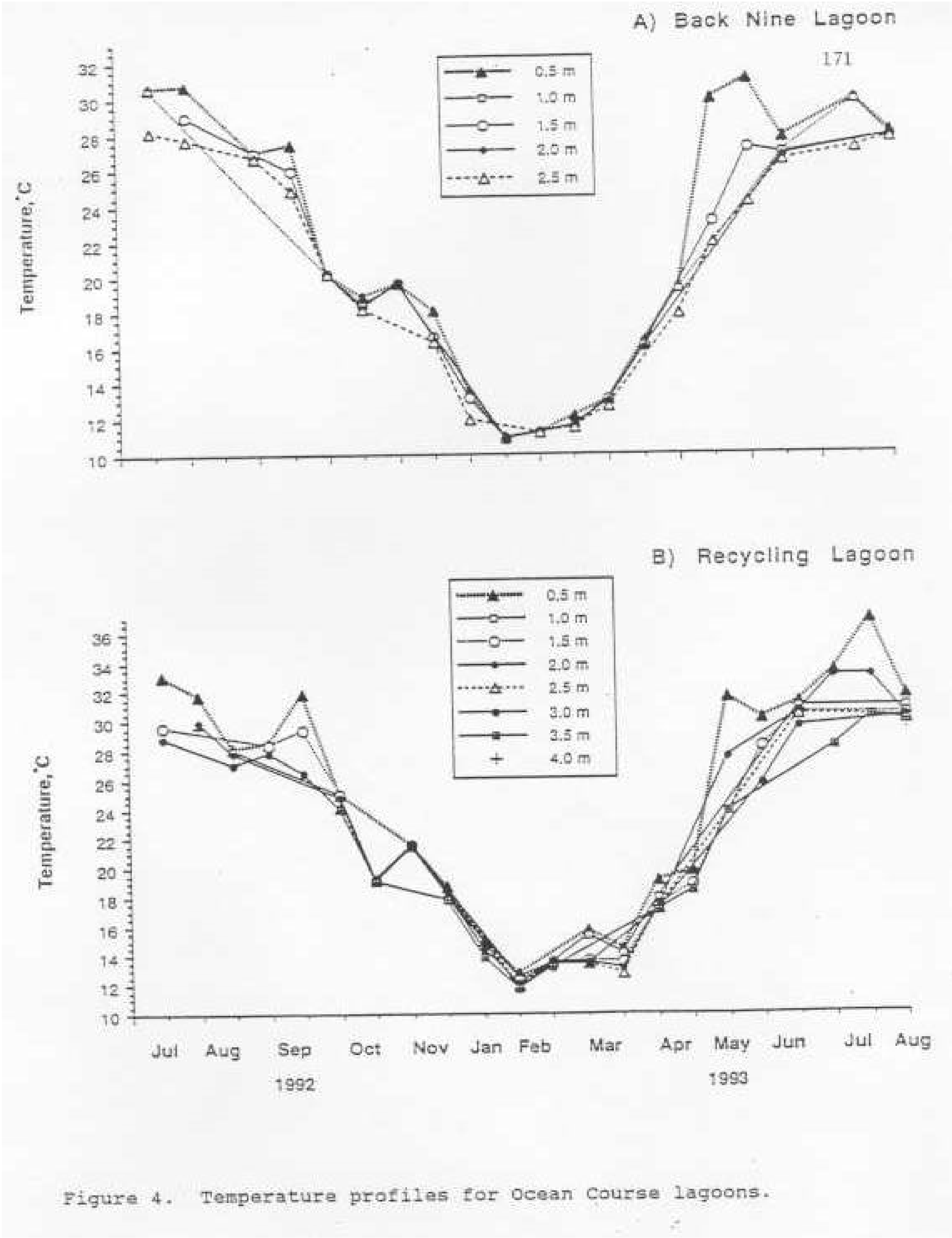

Figure 4. Temperature profiles for Ocean Course lagoons.

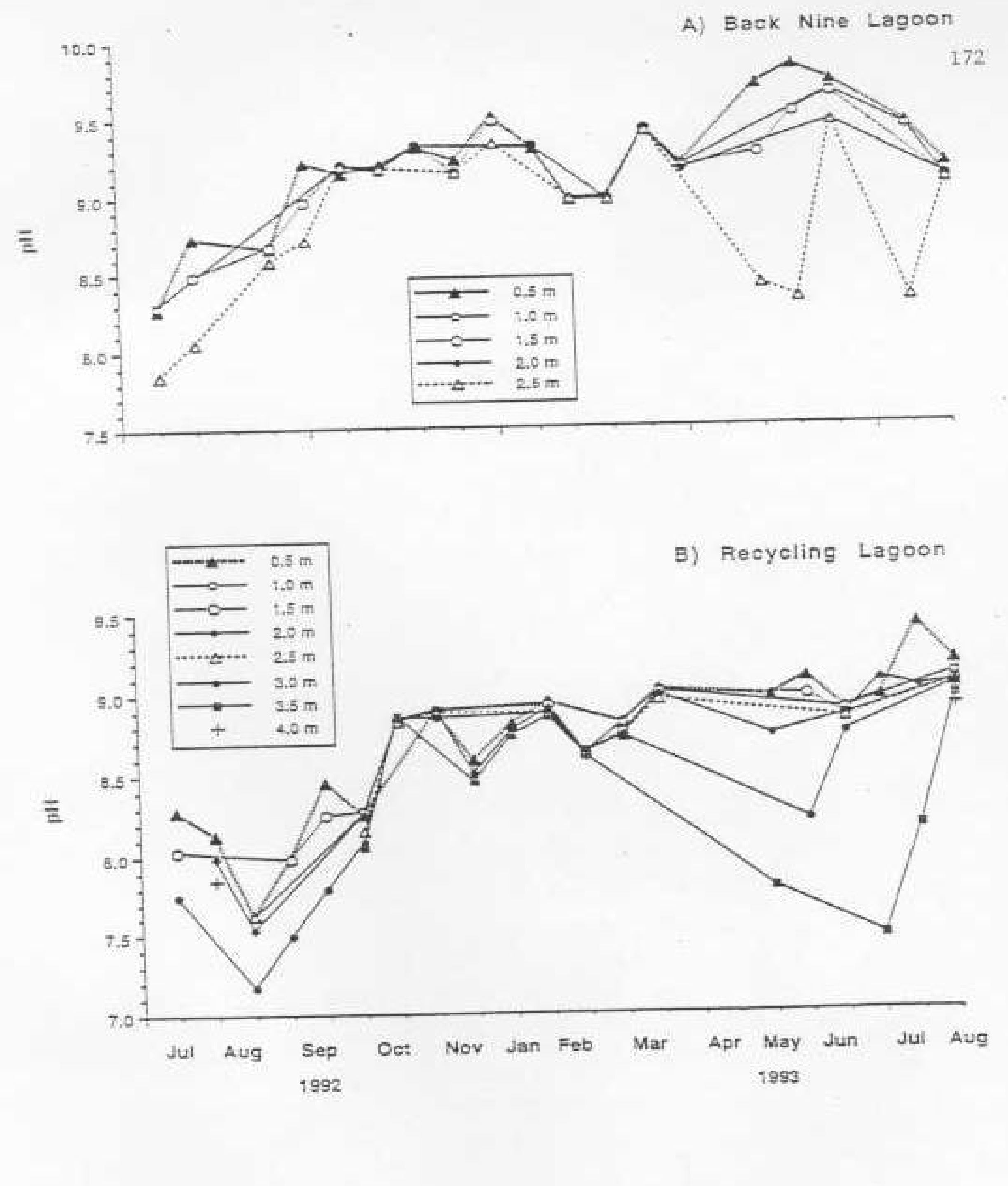

Figure 5. pH profiles for Ocean Course lagoons.

Figure 5. pH profiles for Ocean Course lagoons.

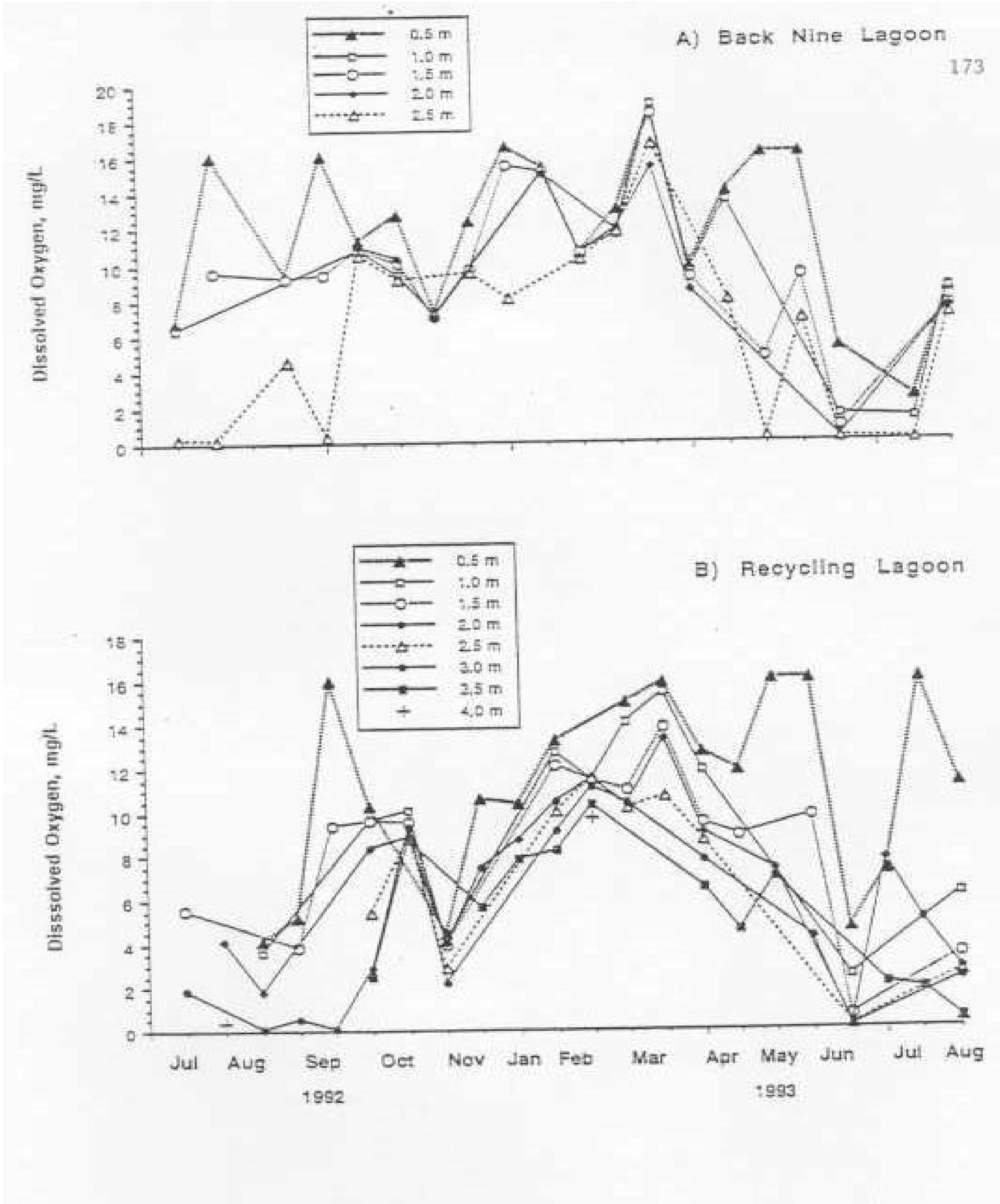

Figure 6. Dissolved oxygen profiles for Ocean Course lagoons.

Figure 6. Dissolved oxygen profiles for Ocean Course lagoons.

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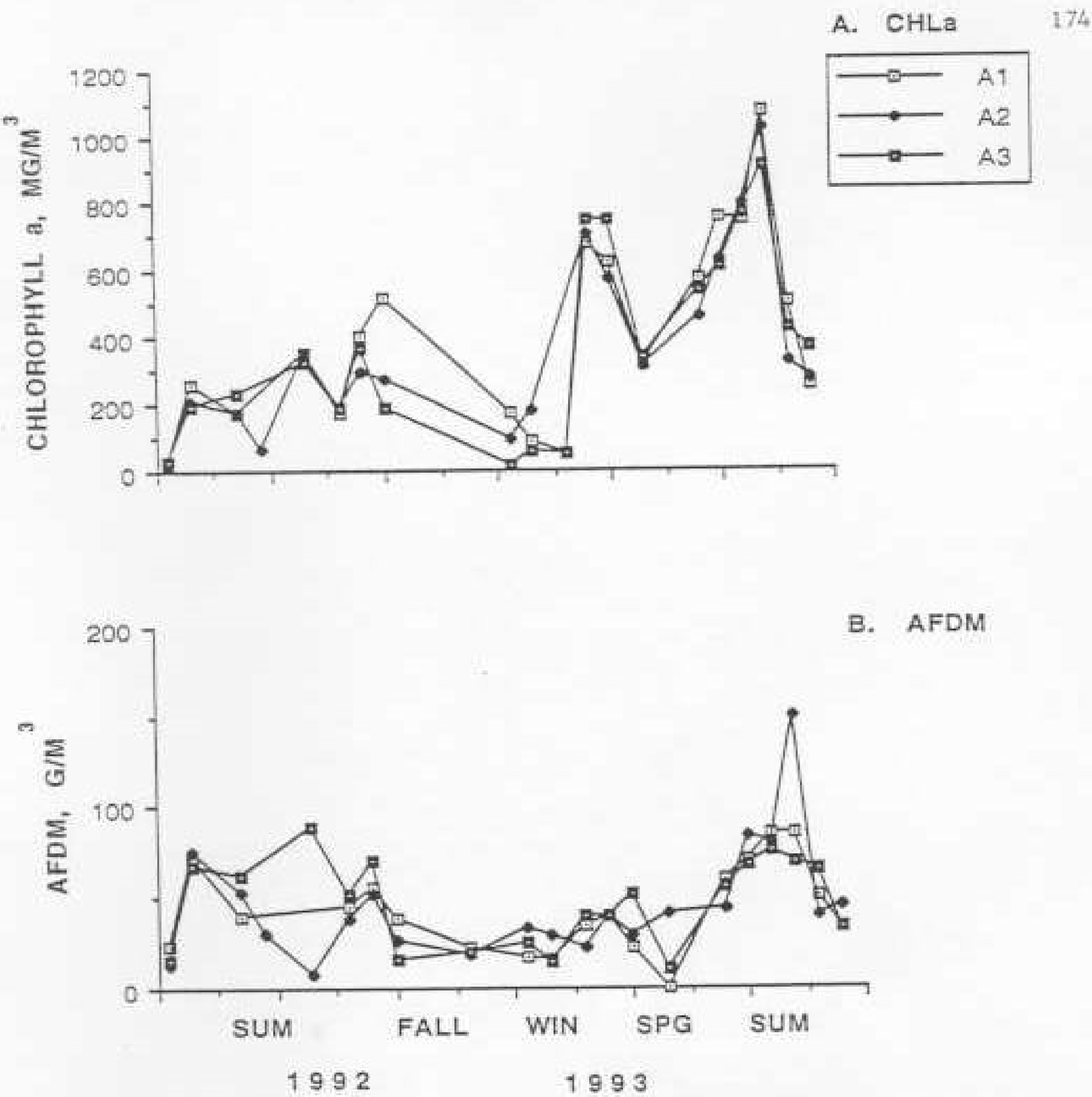

Figure 7. Chlorophyll a and AFDM concentrations in the Ocean Course Back Nine Lagoon.

Figure 7. Chlorophyll *a* and AFDM concentrations in the Ocean Course Back Nine Lagoon.

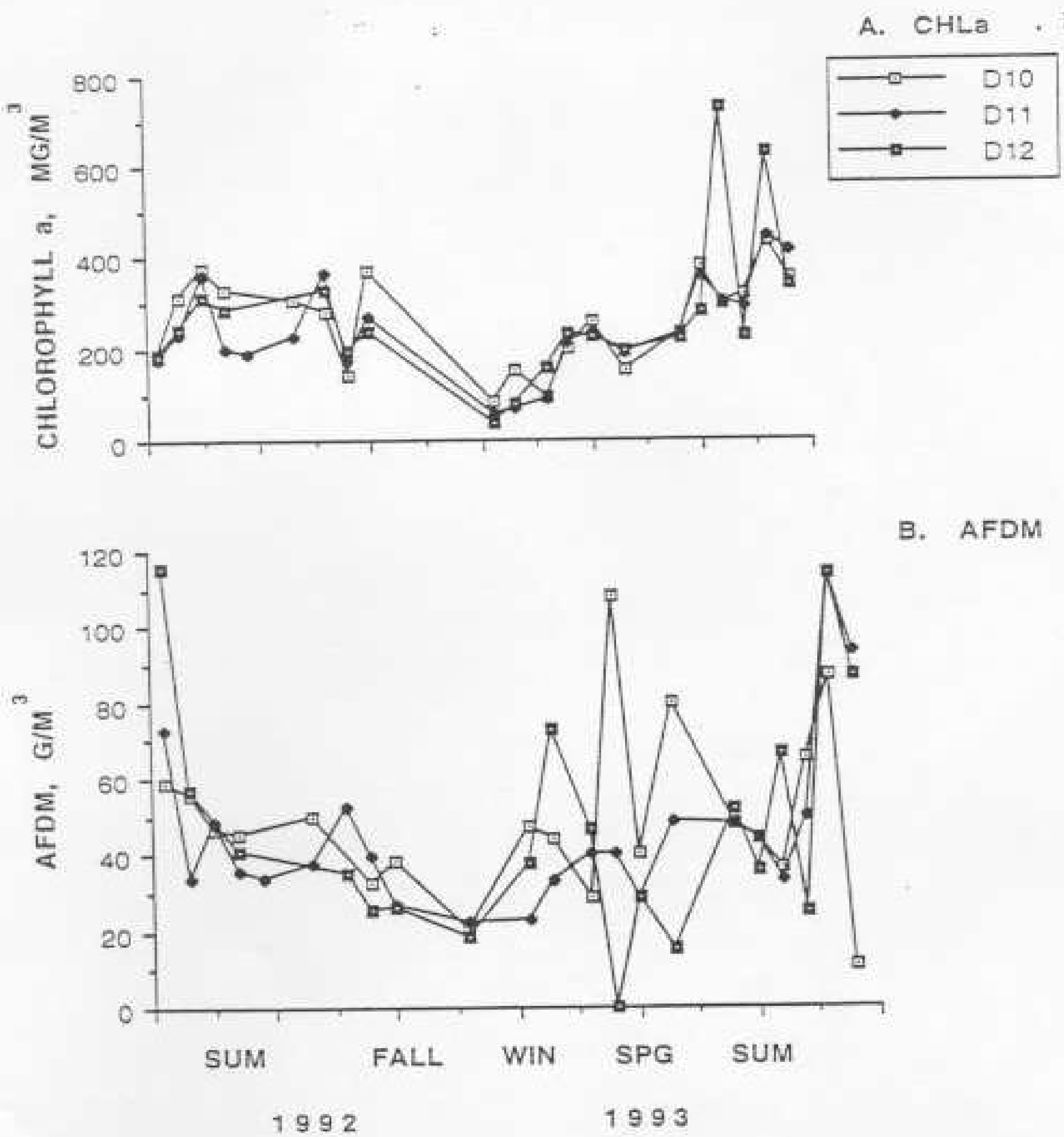

Figure 8. Chlorophyll a and AFDM concentrations in the Ocean Course Recycling Lagoon.

Figure 8. Chlorophyll *a* and AFDM concentrations in the Ocean Course Recycling Lagoon.

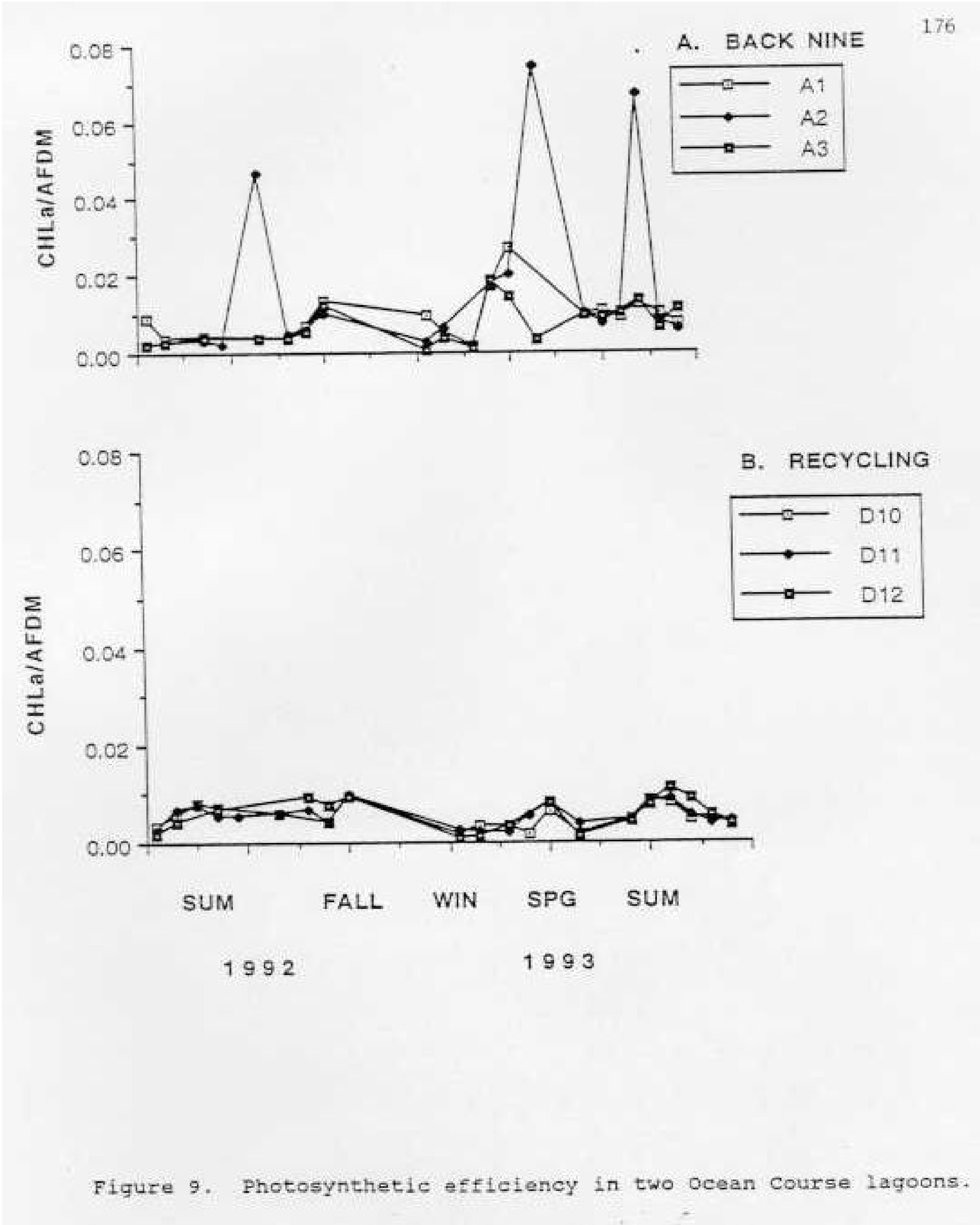

Figure 9. Photosynthetic efficiency in two Ocean Course lagoons.

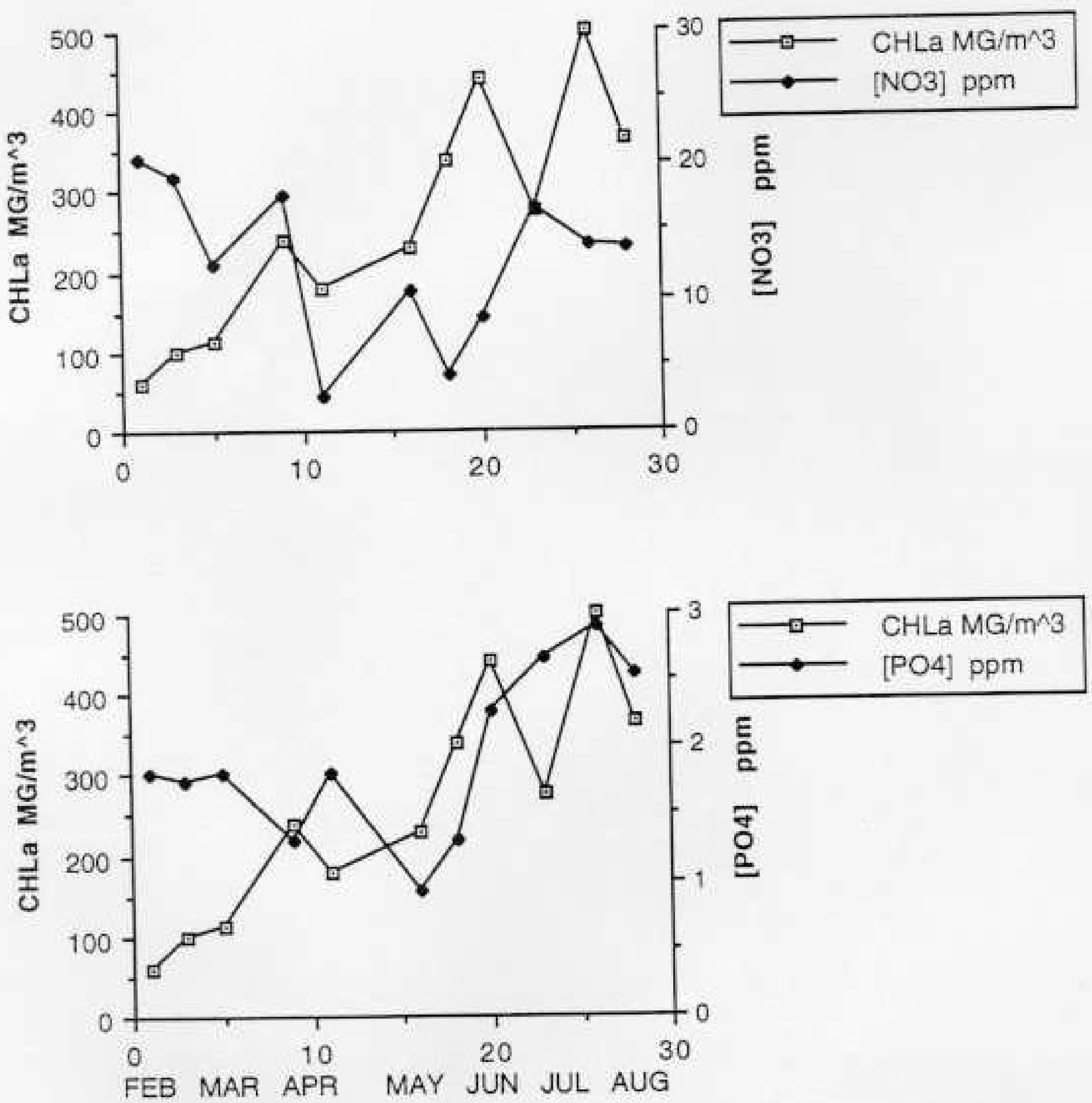

Figure 10. Seasonal relationship of chlorophyll a to nitrate and phosphate concentration in the Ocean Course Back Nine Lagoon.

Figure 10. Seasonal relationship of chlorophyll *a* to nitrate and phosphate concentration in the Ocean Course Back Nine Lagoon.

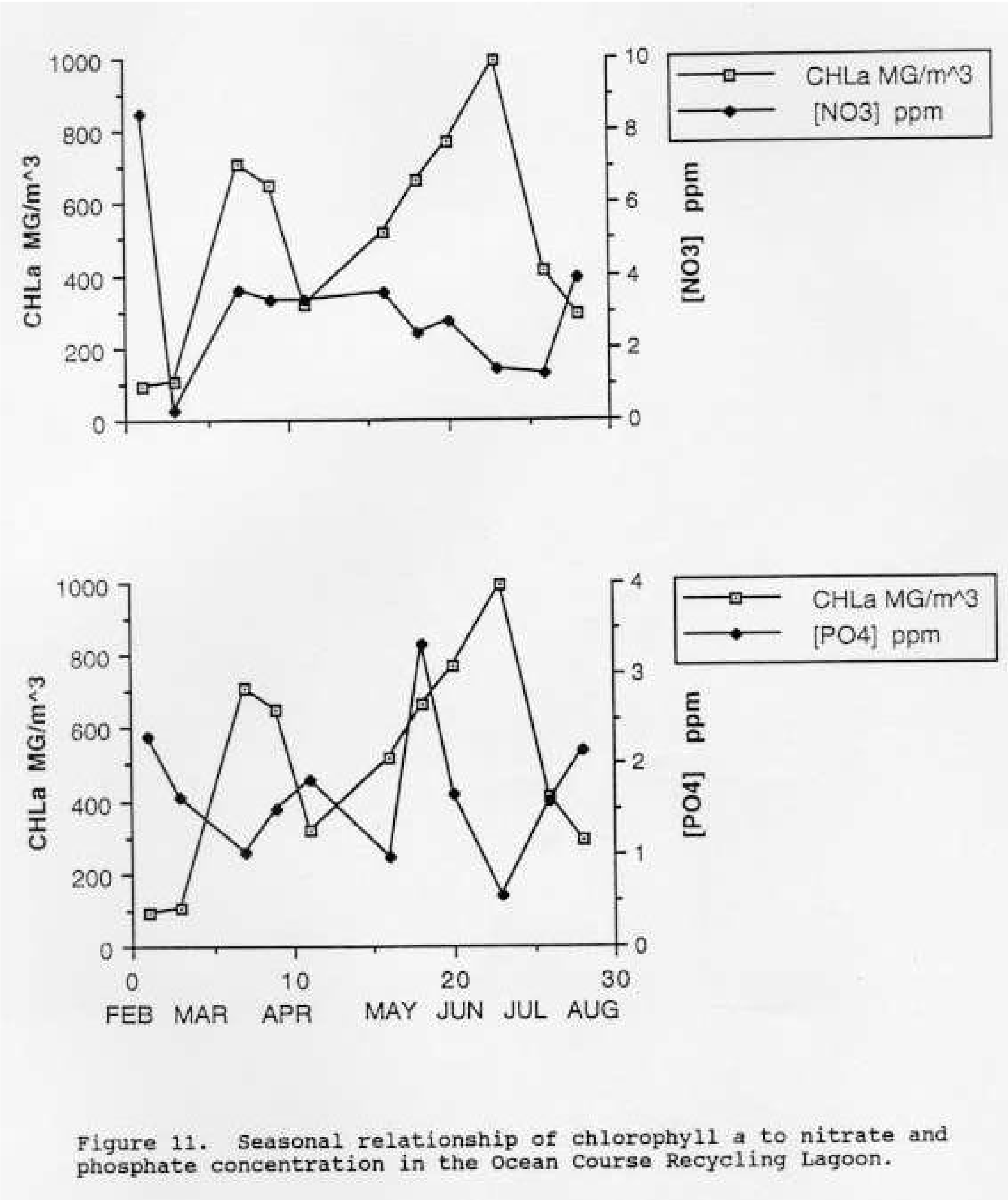

Figure 11. Seasonal relationship of chlorophyll *a* to nitrate and phosphate concentration in the Ocean Course Recycling Lagoon.

CHAPTER 9

MANAGEMENT PRACTICES OF SOUTH CAROLINA GOLF COURSES: A SURVEY OF SUPERINTENDENTS

MANAGEMENT PRACTICES OF SOUTH CAROLINA GOLF COURSES: A SURVEY OF SUPERINTENDENTS

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Abstract - Golf has become an enormously popular activity by millions of Americans. The ever growing interest requires that more golf courses be built and that they be meticulously maintained. The maintenance of a beautiful golf course requires a variety of pesticides be used, as well as fertilizer. Golf courses are managed by people of varied educational backgrounds under a range of budget constraints. The objective of this survey was to identify pesticide use and practices of South Carolina's 336 golf course superintendents. Nearly 30% of the superintendents responded, from 31 of the 46 counties.

Keywords - Golf Management Pesticides Fertilizers

INTRODUCTION

Golf is a multimillion dollar business that requires the use of enormous amounts of pesticides and fertilizers to maintain the well manicured course that is expected by those paying to play. Each course, averaging between 124 and 180 acres [1,2] is managed by a superintendent that is educated in the art of turfgrass maintenance. The turfgrass industry includes not only golf courses but other sports playing fields and home lawns. Of this huge industry, golf only represents approximately 6% of the total money involved [2,3]. Different regions of the country require specific golf course management strategies that have been developed from superintendents' experience and various other sources, including the scientific literature.

Public concerns for the environment and increasingly stringent water quality restrictions have led to the idea of best management practices (BMPs) for golf courses. Krivak [4] conceptualized BMPs for agricultural crops. The BMP concept consist of five basic strategies: 1) decrease the offsite transport of pesticides, nutrients, and sediments, 2) control the application of these chemicals, 3) decrease the total chemical loads, 4) use both biological and mechanical soil and water conservation plans (SWCPs), and 5) educate both the managers and the public about the relationship between environmental issues and golf course management [5]. However, research published to date lacks the ability to provide BMPs to golf course superintendents. There are only a few attempts at such a task [6-9]. The project at the Ocean Course on Kiawah Island is an effort to bring together many of the facets of environmental research into a single holistic approach to providing plausible management strategies. The objective of this survey is to identify the different management strategies used by South Carolina's superintendents for the year 1991.

METHODS

Questions were formulated that were easily understood and answered. Most questions were multiple choice, except for the application rates of pesticides and fertilizers. The topics probed included size, budget, pesticide use, fertilizer use, and training of personnel of each golf course. An initial trail of the survey was sent to several golf courses outside of South Carolina. This was an attempt to measure response time, response rate and any unforeseen difficulties. Following the trial run, some questions were revised and then the survey was sent to 336 superintendents of South Carolina in May of 1992. Reminder notices were sent in June. We began to receive responses in July.

RESULTS AND DISCUSSION

A total of 91 responses were received of the 336 sent. Response was evenly distributed among the three regions of the state (Coastal-27.5%, Midlands-31.8%, Piedmont-39.8%). The average size of each course was 187 acres, with 75% of them being regulation 18 hole courses. More than half of the responding superintendents operated on a budget under $500,000. Of the superintendents responding, 29% held a bachelor degree and 42% had 13-20 years experience. Balogh and Walker (5) have outlined the potential detriments of construction and management of golf courses. Probably the most important concern of the present is the potential for contamination of surface and groundwaters with sediment, nutrients, and pesticides [10-18]. Nearly three quarters of the respondents reported that runoff from the course is not collected for treatment or reuse. Wetlands, ponds or lakes, and riverine systems were the type of habitat receiving the runoff from 21, 67, and 40% respectively, of the courses surveyed. Obviously, there is the concern of possible negative impacts on nontarget organisms by existing chemical management strategies [19-22]. The U.S. is not the only place where public awareness has brought this to the attention of governmental agencies. Japan has begun setting standards for maximum allowable pesticide residues found in golf course effluent [23]. Only 7.8% of the responding superintendents reported to have had a fish or wildlife kill on their course in the first five months of 1992. Eighty-three percent spent less than $24,000 in 1992 for pesticide purchases. More than 70% of the superintendents rated applicator, golfer and environmental safety as highly influencing their use of pesticides. Nearly all were willing to ask an extension officer or some other qualified person about the best method to solve a pest problem. A wide variety of pesticides were used by the superintendents responding to the survey (Table 1). Chlorpyrifos, glyphosate, chlorothalonil and fenamiphos were the most commonly used insecticide, herbicide, fungicide and nematicide respectively.

In order to maintain the lush green carpet of turfgrass, an extremely large quantity of water is used. The push for water conservation has resulted in increased utilization of secondary treated sewage effluent for irrigation [24,25]. Only a small percentage (less than 10%) of the courses use recycled effluent for irrigation water. Hence, they rely much more heavily on fertilizer applications. Fertilizer management poses some concerns similar to those associated with pesticides. Petrovic [16] summarizes and reviews the literature dealing with nitrogenous fertilizer usage. Nitrogen and potassium fertilization of turfgrass has been linked to the ability to survive stress conditions brought on by disease, drought, or human foot traffic [26-28]. Granular forms of fertilizer are most commonly used.

In conclusion, much work still has to be done in order to present golf course superintendents with a "cookbook" approach to pest management. An interdisciplinary team consisting of superintendents, agronomists, environmental toxicologists, and chemists are needed to review past and current strategies. From this intensive cooperation, future research and strategies may be formed.

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Table 1. Pesticides used on golf courses in South Carolina in 1992

Insecticides	Herbicides	(Herbicides, cont.)	Fungicides	Nematicides
Acephate	Atrazine	Prodiamine	Anilazine	Ethoprop
Amidinohydrazone	Benefin	Pronamide	Benomyl	Fenamiphos
Carbaryl	Benefin + Oryzalin	Sethoxydim	Chloroneb	
Diazinon	Benefin + Trifluralin	Simazine	Chlorothalonil	
Ethoprop	Bensulide	Siduron	Chlorothalonil + Fenarimol	
Fenoxycarb	Bensulide + Oxadiazon		Ethazole	
Fonofos	Bentazon		Fenarimol	
Fluvalinate	Bromoxynil		Foscetyl	
Isazofos	CAMA		Iprodione	
Isofenphos	2,4-D		Maneb	
Pyrethrin	2,4-D + Dichlorprop		Mancozeb	
Trichlorfon	2,4-D + MCPP + Dicamba		Metalaxyl	
1,1,1,Trichloroethane	DCPA		Metalaxyl + Mancozeb	
	Fenoxaprop-ethyl		PCNB	
	Glyphosate		Propiconazole	
	Imazaquin		Propamocarb	
	Isoxaben		Thiophanate	
	MCPP + 2,4-D		Thiophanate-methyl	
	Methyl Bromide		Thiophanate-methyl + Mancozeb	
	Metolachlor		Thiophanate-methyl +Iprodione	
	Metribuzin		Thiram	
	Metsulfuron-methyl		Triadimefon	
	MSMA		Vinclozolin	
	Oryzalin			
	Oxadiazon			
	Oxadiazon + Benefin			
	Dicamba			
	Dicamba + 2,4-D			
	Diclofop-methyl			
	Dithiopyr			
	DSM			
	Ethofumesate			
	Fenarimol			
	Paraquat			
	Pendimethalin			

S U M M A R Y

SUMMARY

The Kiawah Island Ocean Course, the site of our model study, is a unique area supporting a diverse array of wildlife. Of great concern on the course was the diversity and abundance of bird utilization and the attention that they attract from both golf course operations as well as the public. One major objective of our project was to focus our sampling of the course around periods of significant bird utilization and pesticide application, particularly in the summer of 1993. Hundreds of birds were seen on the course over a period of several months during the summer of 1993. Potential exposure to birds associated with pesticide application appeared quite high. During the course of our study only one impaired bird, a laughing gull (*Larus atricilla*), was observed after application of Turcam7 at 3.36 kilogram active ingredient per hectare. This bird exhibited 87% depression of total cholinesterase, and 50.33 micrograms active ingredient were recovered in foot wash samples. Intensive monitoring of other birds that highly utilize the turf, including common grackles (*Quiscalus quiscula*), boat-tailed grackles (*Quiscalus major*), and red-winged blackbirds (*Agelaius phoeniceus*) suggested non-significant depression of total cholinesterase activity following pesticide applications. As the golf course ages and more intensive pesticide application may be required, particularly for the control of mole crickets, further monitoring of birds will be in order. Avian exposure to pesticides on golf courses has been identified in several cases by TIWET scientists [1,2]. Negative impacts to birds on golf courses could result in serious public backlash. Non-lethal biochemical assessment of avian exposure to pesticides has been worked out in TIWET laboratories and can be definitively applied toward avian field studies. This work will have significant importance in future bird assessment studies, particularly since sacrifice of birds in field studies will receive more scrutiny in the future.

The environmental fate assessment of pesticides on golf courses is critical to the understanding of potential exposure to fish and wildlife. To evaluate the environmental fate of pesticides applied to golf courses, soil columns were utilized in TIWET laboratories. Artificial soil columns were prepared with soil mixtures that simulated a golf course "putting green" on the Ocean Course, Kiawah Island, South Carolina. Both chlorpyrifos and bendiocarb mobility through root zone mixtures using the constructed soil columns were examined. Limited movement of chlorpyrifos through either soil mix with or without turf was observed. Bendiocarb migration was significantly reduced by turf/thatch in soil. Bendiocarb should pose minimal risk to the surrounding ecosystems as long as it is only applied to turf and care is maintained to not overspray sand traps and surrounding dunes. This was due to the fact that bendiocarb would migrate through root zone mixes but not turf in the soil column test. These types of assessments can help prevent potential movement of unwanted pesticide residues into the golf course environment. By becoming aware of movement characteristics utilizing simulated approaches, as presented with soil columns, one can do a great deal to assess potential environmental fate of pesticides in managing a golf course.

In actual field simulations, bendiocarb was applied to the turf at the Ocean Course on Kiawah Island, South Carolina, and monitored for its environmental chemistry. After application, turf was found to contain over 500 nanograms bendiocarb per gram. The decay profile was extremely rapid and by four days post application, bendiocarb was not observed in turf patches. For this reason, environmental exposure to bendiocarb would be limited to probably one day and perhaps into the second day post application. This would be particularly important for birds that might feed on turf, or in the case of waterfowl such as widgeon that actually might consume turf for food. Bendiocarb that did enter into the water was present in aquatic plants sampled. Levels in the plants increased following known applications of bendiocarb. In the present study, 400 nanograms per gram in plant tissue was not exceeded in terms of bendiocarb residues. This quantity for ecologically relevant levels in plants is relatively small.

Perhaps the most significant ecologically relevant factor facing the Ocean Course at Kiawah Island was the enhancement of vegetation growth on the golf course with intensive fertilizer application. The Ocean Course at Kiawah Island utilizes an irrigation water recapture system which minimizes potential nutrient loading into adjoining brackish marshes. Measurement of water quality through chlorophyll *a* concentrations into lagoons in the recycling system indicates a highly productive system. Levels of chlorophyll *a* over 1000 milligrams per cubic meter and biomass over 110 grams per cubic meter were measured. Nutrient analyses for nitrate and phosphate indicated levels as high as 21 parts per million and 3.8 parts per million respectively, and would direct ones concern to the potential for off-site eutrophication. Nutrients in the Ocean Course lagoons indicate the movement of nutrient rich runoff into the lagoons and ultimately into Willet Pond. There is the potential for eutrophication to occur in Willet Pond and should be closely monitored. To-date we have neither observed nor received any reports of adverse affects within the pond.

Golf course management practices on the Ocean Course, Kiawah Island, at this time utilizes various organophosphate and carbamate pesticides such as acephate (Orthene**7**), chlorpyrifos (Dursban**7**) and bendiocarb (Turcam**7**). As has been discussed, a significant amount of fertilizer is also applied to the course. To evaluate the potential for runoff of these materials into the aquatic system and the impact to fish, sheepshead minnows and mosquitofish which are found in the courses lagoon system were evaluated. Laboratory acute toxicity tests were performed with chlorpyrifos and bendiocarb insecticides. Using sheepshead minnows as an indicator species, brain cholinesterase activity was used to compare the response of fish dosed in the laboratory and that of those exposed to bendiocarb on the course via a artificially induced worse case scenario. Present data suggests that neither the use of Dursban**7** (chlorpyrifos) or Turcam**7** (bendiocarb) on the Ocean Course at current label rates would pose risk to these species. Further field assessment work would be in order to further define these exposure parameters and potential impact.

As far as the overall management practices on South Carolina golf courses, the use of pesticides and fertilizers are extremely important. A survey of superintendents indicated that the array of insecticides, herbicides, fungicides, and nematicides used on golf courses is diverse. One of the most widely used insecticides on South Carolina golf courses is chlorpyrifos which in terms of its evaluation in the Ocean Course, Kiawah Island study appears appropriate.

The USGA Environmental Research Committee requested an integrated study to evaluate both pesticides and nutrient input into a golf course ecosystem and begin to assess potential impacts on fish and wildlife resources. As of three years ago, this had not been done and to-date we are not aware of a similar integrated ecosystem study. Although we will agree that the number of variables measured and products evaluated is limited when considering an ecosystem level study, we believe this model effort is moving in the right direction. The use of insecticides on golf courses can pose a hazard to birds and other wildlife. This should be carefully evaluated and monitored. We saw evidence of some intoxication, but this was limited. In terms of ecological risk, the use of nutrients may pose a far greater impact situation. At the Ocean Course the high utilization of fertilizers and the runoff associated in the collection in the lagoon system revealed a highly productive system that was moving toward "eutrophication." This process can result in diminishment of water quality and the appearance of unsightly blue-green algal mats, depressing oxygen in the water available to fish species.

Surface water contamination and movement of pesticide residues in the turf was limited. The soil column studies verified the physical limits and environmental chemical characteristics of two particular products, chlorpyrifos and bendiocarb on soil column cores, representing the Kiawah Island system. Simple tests such as this could reveal the potential for movement of pesticide residues after application. This is particularly important with the potential for fish and/or bird kills attracting so much negative public attention.

Additional work at the Ocean Course, Kiawah Island should continue to track water quality, particularly the water quality in the lagoon system and the potential to move off-site to Willet Pond. In addition, birds should be monitored carefully, particularly during the period of year when heaviest insecticide applications are utilized. Sub-lethal monitoring techniques such as assessment of plasma cholinesterase and documented evidence of pesticide exposure via footwash samples analyzed by HPLC (high performance liquid chromatography) is extremely useful. This work will continue to better define the potential for avian exposure to pesticide residues utilized on golf courses.

REFERENCES

1. **KENDALL, R.J., L.W. Brewer,** and **R.R. Hitchcock.** 1993. Response of Canada Geese to a turf application of diazinon AG500. J. Wildl. Dis. **29**(3):458-464.

2. **KENDALL, R.J., L.W. Brewer, R.R. Hitchcock,** and **J.R. Mayer**. 1992. American widgeon mortality associated with turf application of diazinon AG500. J.Wild. Dis. **28**(2):263-267.

A P P E N D I C E S

TABLE OF APPENDICES

* *This appendix is not included. To obtain this data, please contact OCRM at (843) 744-5838; 1362 McMillan Avenue, Suite 400, Charleston, SC 29405*

EXECUTIVE SUMMARY - APPENDIX 1

Common names, trade names, generic names and action of pesticides

Appendix 1. Common names, trade names, generic names and action of pesticides mentioned in this report (Chapters 1-8).

COMMON NAME	TRADE NAME	GENERIC NAME	ACTION
Acephate[a]	Orthene	O,S-dimethyl acetylphosphoramidothioate	insecticide
Atrazine[b]	G-30027, Malermais, X-siprim, Vegfru Solaro, Farmco Atrazine, Gesaprim, Zeaphos	2-chloro-4-ethylamino-6-isopropylamino-s-triazine	herbicide
Bendiocarb[a]	Turcam, Dycarb, Ficam, Garvox, Multamat, NC6897, Niomil, Seedox, Tatoo	2,2-dimethyl-1,3-benzodioxol-4-yl methylcarbamate	insecticide
Carbofuran[a]	Furadan	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate	insecticide, nematicide
Chlorpyrifos[c]	Dursban, Lorsban	O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate	insecticide
Diazinon[a]	Spectracide, AG500, Alfa-tox, Sarolex, D-Z-N Diazinon 14G	O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl phosphorothioate	insecticide
Dicrotophos[d]	Bidrin, Carbicron, Ektafos	3-(dimethoxyphosphinyloxy)-N,N-dimethyl-*cis*-crotonamide	insecticide
Fenamiphos[b]	Nemacur	Ethyl 3-methyl-4-(methylthio)phenyl (1-methylethyl) phosphoramidate	nematicide
Fenitrothion[a]	Bayer 41831; Bayer S-5660; Bayer S-1102A; AC-47, 300; C 17114; Accothion; Cytel; Cyfen; Folithion; Sumithion; Agrothion; Dicofen; Fenstan; Metathion E-50; Verthion; Cekutrothion; Dybar; Fenitox; Novathion; Nuvanol	O,O-dimethyl O-(4-nitro-m-tolyl) phosphorothioate	insecticide/acaricide
Fenthion[a]	Baytex, Entex, Bayer 29493, Bayer s-1752, Baycid, Lebaycid, Spotton, Tiguvon, Mercaptophos	O,O-dimethyl-O-[4-(methylthio)-m-tolyl] phosphorothioate	insecticide/ acaricide, avicide

Appendix 1. (Continued, p. 2)

COMMON NAME	TRADE NAME	GENERIC NAME	ACTION
Glyphosate[a]	Roundup, Rodeo, Roudup L&G, Polado, Shackle, Shackle C	N(phosphonomethyl) glycine	herbicide
Imazaquin[e]	Image	Ammonium salt of 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidizol-2-yl]-3-quinoline	herbicide
Isazophos[c]	Triumph, CGA-12223	O-(5-chloro-1-[methylethyl]-1H-1,2,4-triazol-3-yl) O,O-diethyl phosphorothioate	insecticide
Malathion[a]	Calmathion, Celethion, Cythion, Chemathion, Malaspray, Detmol MA 96%, Emmatos, Emmatos Extra, For-Mal, Fyfanon, Hilthion, Karbofos, Kop-Thion, Kypfos, Malamar, Malaphele, Malathion ULV Concentrate, Malatol, Maltox Spray, Sumitox, Vegfru Malatox, Zithiol, Malmed	O,O-dimethyl phosphorodithioate of diethyl mercaptosuccinate	insecticide, miticide
Methoprene[b]	Altosid SR-10, Altosid Briquets, ZR-515	Isopropyl (2E-4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate	insecticide
Methyl parathion[c]	Parathion-methyl, Metaphos, Cekumethion, Devithion, Dimethyl parathion, E601, Folidol M, Fosferno M50, Parataf, Paratox, Patron M, Penncap M, Tekwaisa, Wofatox, Metacide, Bladan M, Metron, Dalf, Nitrox 80	O,O-dimethyl-O-4-nitrophenyl phosphorothioate	insecticide
MSMA[b]	Ansar 6.6, Herb-All, Merge 823, Target MSMA	Monosodium methanearsonate	herbicide
Paraquat[b]	Herbaxon, Cekuquat, Dextrone, Paquat, Herboxone, Pillarxone, Pillarquat, Total, Toxer	1,1-Dimethyl-4,4-bipyridinium ion	herbicide

Appendix 1. (Continued, p. 3)

COMMON NAME	TRADE NAME	GENERIC NAME	ACTION
Parathion[c]	Alkron, Alleron, Aphamite, Bladan, Corothion, Ethyl parathion, Folidol E-605, Fosferno 50, Niran, Orthophos, Panthion, Paramar, Paraphos, Parathene, Parawet, Phoskil, Rhodiatox, Soprathion, Stathion, Thiophos	O,O-diethyl-O-p-nitrophenyl phosphorothioate	insecticide
PCP[b]	Pentacon, Penwar, GLAZD Penta and Block Penta, Penchlorol, Sinituho	Pentachlorophenol	wood preservative, molluscicide

[a]U.S. Environmental Protection Agency. 1990. Pesticide Fact Handbook, Volume 2. Noyes Data Corporation, Park Ridge, NJ, U.S.A.

[b]Farm Chemicals Handbook. 1993. Meister Publishing Company, Willoughby, OH.

[c]U.S. Environmental Protection Agency. 1988. Pesticide Fact Handbook, Volume 1. Noyes Data Corporation, Park Ridge, NJ, U.S.A.

[d]**Budavari**, **Susan**, editor. 1989. The Merck Index: An encyclopedia of chemicals, drugs, and biologicals. Eleventh ed. Merck & Co., Inc. Rahway, NJ, U.S.A.

[e]Material Safety Data Sheet. 1987. American Cyanimid Company, Wayne, NJ.

EXECUTIVE SUMMARY - APPENDIX 2

Presentations related to the Kiawah Island research at the 14th Annual Meeting of the Society of Environmental Toxicology and Chemistry, November 14-18, 1993, in Houston, Texas.

MOVEMENT OF CHLORPYRIFOS AND BENDIOCARB
THROUGH TWO SOIL MATRICES

CHAPTER 5

APPENDIX 1

TIWET/SOP 401-27-02: Extraction of soil for volatile organophosphate pesticide determination by GC/FPD analysis

MOVEMENT OF CHLORPYRIFOS AND BENDIOCARB
THROUGH TWO SOIL MATRICES

CHAPTER 5

APPENDIX 2

Turf Diagnostics report on root zone characteristics

EFFECTS OF NUTRIENT LOADING ON A SOUTH CAROLINA GOLF COURSE

CHAPTER 8

APPENDIX 1

Nutrient levels in Kiawah Island Ocean Course waters

EFFECTS OF NUTRIENT LOADING ON A SOUTH CAROLINA GOLF COURSE

CHAPTER 8

APPENDIX 2

Water quality parameters for back nine and recycling lagoons

EFFECTS OF NUTRIENT LOADING ON A SOUTH CAROLINA GOLF COURSE

CHAPTER 8

APPENDIX 3

Chlorophyll *a* levels measured for the back nine and recycling lagoons